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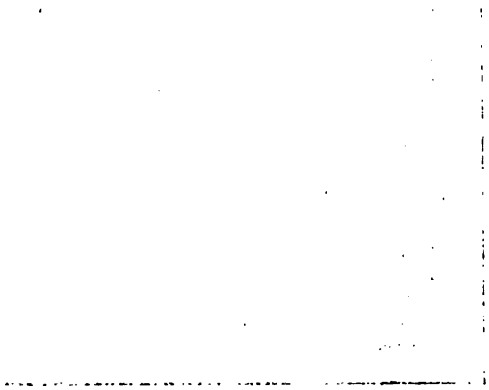

WORKS OF

PROFESSOR J. A. MANDEL

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TRANSLATIONS

A Text-book of Physiological Chemistry.

By Olof Hammarsten, Emeritus Professor of Medical and Physiological Chemistry in the University of Upsala, with the Collaboration of S. C. Hedin, Professor of Medical and Physiological Chemistry in the University of Upsala. Authorised translation from the Author's enlarged and revised 8th German edition, by John A. Mandel, Sc.D., Professor of Chemistry in the New York University and Bellevue Hospital Medical College. viii + 1026 pages. 6 by 9. Cloth, $4.00 net.

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A TEXT-BOOK

OF

PHYSIOLOGICAL CHEMISTRY

BY

OLOF HAMMARSTEN

EMERITUS PROFESSOR OF MEDICAL AND PHYSIOLOGICAL CHEMISTRY IN THE UNIVERSITY OF UPSALA

WITH THE COLLABORATION OF

S. G. HEDIN

PROFESSOR OF MEDICAL AND PHYSIOLOGICAL CHEMISTRY IN THE UNIVERSITY OF UPSALA

Authorized Translation

FROM THE AUTHOR'S ENLARGED AND REVISED EIGHTH GERMAN EDITION

BY

JOHN A. MANDEL, Sc.D.

PROFESSOR OF CHEMISTRY IN THE NEW YORK UNIVERSITY AND BELLEVUE HOSPITAL MEDICAL COLLEGE

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16

PREFACE TO THE EIGHTH GERMAN EDITION

THE revision of this edition has been accomplished with the collaboration of Professor S. G. Hedin, and the work has been divided so that Hedin has revised Chapters I, III, VIII, XII and XVI, besides the Index of Authors, while Hammarsten has revised Chapters II, IV, V, VI, VII, IX, X, XI, XIII, XIV, XV, and XVII, besides the General Index. The numerous recent developments in physiological chemistry have made a thorough revision and reconstruction necessary in all the chapters, and in order to prevent a noticeable increase in the size of the work, it was also necessary to change the arrangement of the foot-notes more economically The number of chapters in this edition is XVII instead of XVIII as in the seventh edition, because for several reasons it was found advisable to combine the first two chapters of the seventh edition into one chapter, and at the same time certain parts of the first chapter have been incorporated into other chapters, thus for example the oxidation processes have been introduced into Chapter XVI (on respiration and oxidation). In general the plan of the work remains unchanged.

OLOF HAMMARSTEN.

UPSALA, September, 1913.

TRANSLATOR'S PREFACE TO THE SEVENTH AMERICAN EDITION

WORKERS in Biochemistry are to be congratulated on the appearance of a new edition of Hammarsten's "Physiologischen Chemie." At this time, when so many new and important biochemical facts are being published and when so many older theories and deductions are found more or less erroneous, due to recent investigations using, new methods, it is very fortunate that we have this complete and critical compilation from the master hand of Professor Hammarsten, now in his 73d year. We all owe him a great debt of gratitude for his painstaking work for so many years.

I take great pleasure in expressing my indebtedness to my assistant, Dr. A. O. Gettler, for the help he has given me in revising the proof and for making the Indexes.

JOHN A. MANDEL.

NEW YORK, June, 1914.

CONTENTS

CHAPTER I

PAGE

General and Physico-chemical 1

CHAPTER II

The Proteins 77

CHAPTER III

The Carbohydrates 196

CHAPTER IV

Animal Fats and Phosphatides 232

CHAPTER V

The Blood 250

CHAPTER VI

Chyle, Lymph, Transudates and Exudates 345

CHAPTER VII

The Liver 381

CHAPTER VIII

Digestion 451

CHAPTER IX

PAGE
TISSUES OF THE CONNECTIVE SUBSTANCE 514

CHAPTER X

THE MUSCLES 565

CHAPTER XI

BRAIN AND NERVES 604

CHAPTER XII

ORGANS OF GENERATION 620

CHAPTER XIII

THE MILK 643

CHAPTER XIV

THE URINE 672

CHAPTER XV

THE SKIN AND ITS SECRETIONS 837

CHAPTER XVI

RESPIRATION AND OXIDATION 850

CHAPTER XVII

METABOLISM 878

INDEX TO AUTHORS 945
GENERAL INDEX 983

PHYSIOLOGICAL CHEMISTRY.

CHAPTER I.

GENERAL AND PHYSICO-CHEMICAL.

I. OSMOTIC PRESSURE.

WHEN certain substances are placed in contact with water they dissolve therein and finally a liquid is obtained which contains an equal quantity of the dissolved substance in each unit volume. There exists between the water and the soluble body a certain attractive force. Upon this force depends also the so-called diffusion, which manifests itself when two different solutions of the same or different substances are brought into immediate contact with each other. The dissolved molecules and the water intermingle with each other so that finally the dissolved bodies are equally divided in the entire quantity of water. Imagine a cane-sugar solution in contact with pure water; the equilibrium or the homogeneity of the system can then be brought about in two ways; namely, the sugar molecule can migrate in part into the water, and secondly, the water can pass into the solution. If the two fluids at the beginning are in immediate contact with each other then the two processes take place simultaneously.

The conditions change when the two liquids are separated from each other by a membrane, which allows of the passage of water but not of the dissolved substance (in this case cane-sugar). In the presence of such a so-called semipermeable membrane the equilibrium can only be established by the water passing into the cane-sugar solution. Semipermeable membranes have been artificially prepared, and they also occur in nature, or conditions exist which give results like those of the membranes. To the first group belong TRAUBE'S so-called precipitation membranes.[1] Such a membrane, for example can be produced by carefully dropping a concentrated solution of copper sulphate into a dilute

[1] Arch. f. (Anat. u.) Physiol., 1867, pages 87 and 129.

solution of potassium ferrocyanide. Thereby the drop of copper sulphate is surrounded by a membrane of copper ferrocyanide, which is impervious to copper sulphate as well as to potassium ferrocyanide, but allows water to pass. The drops retain their blue color in the yellow solution but increase in volume, due to the taking up of water, until the tension of the membrane prevents the further increase in size. If the difference in concentration of the two solutions is great enough, the membrane is ruptured by the pressure.

In order to give the copper-ferrocyanide membrane a greater rigidity, PFEFFER has suggested forming the precipitate on a porous, rigid wall.[1] For this purpose he makes use of a small, porous earthenware cell which, after careful cleaning, is treated with copper sulphate and potassium ferrocyanide so that the membrane is precipitated on the inner wall of the cell. The membrane thus obtained is impervious to the cane-sugar. If the cell is filled with a cane-sugar solution and then placed in pure water, no sugar leaves the cell, while water passes into the cell, and this continues until the opposite pressure produced prevents the further passage of water. If the cell is completely closed and in connection with a manometer, then on the establishment of an equilibrium the manometer indicates the force with which the inclosed solution attracts water.

As the sugar is attracted with the same force by the water as the water is by the sugar and also as the sugar cannot pass through the membrane therefore the sugar exerts a pressure upon the membrane equal to the pressure indicated by the manometer. This pressure is called the *osmotic pressure* of the enclosed solution. For dilute cane-sugar solutions PFEFFER'S determinations show that the osmotic pressure is approximately proportional to the concentration and slowly rises with the temperature.

Experiments with other semipermeable membranes have also been carried out by DE VRIES, and these will be discussed on page 5. DE VRIES' experiments have led to the following result: *Solutions of analogously constructed bodies having the same molecular concentration give the same osmotic pressure.*

VAN'T HOFF first called attention to the analogy which exists between the laws of osmotic pressure of a dissolved substance and of gases,[2] namely, that the osmotic pressure is proportional (or inversely proportional to the volume of the solution) to the concentration, and corresponds completely with BOYLE-MARIOTTE'S law on the relation between the volume and pressure of gases. Also, that equimolecular solutions

[1] Osmotische Untersuchungen, Leipzig, 1877.
[2] Zeitschr. f. physik. Chem., 1, 481 (1887).

have the same osmotic pressure, corresponds to AVOGADRO'S law, that equal volumes of different gases under the same pressure contain the same number of molecules.

From PFEFFER'S results of the osmotic pressure of cane-sugar solutions VAN'T HOFF has calculated that it is the same as the pressure exerted by any gas of the same molecular concentration and temperature. In general the following is true:

Dissolved bodies exert in solution the same osmotic pressure they would exert if they were gases at the same temperature and in equal volume.

Recently MORSE, FRAZER and collaborators have brilliantly substantiated the theory of VAN'T HOFF for solutions of cane-sugar and glucose, by making use of PFEFFER'S method but using a very refined technique.[1]

From what has been given, the osmotic pressure of a solution, separated from the surrounding pure solvent by a semipermeable membrane, exerts its effects in two ways. First the pure solvent tries to enter the solution and secondly the dissolved substance presses upon the membrane with a force equal to the gas pressure. According to whether we consider either one or the other of these ways, the osmotic pressure of a solution can be considered as its ability to attract the solvent, or as a pressure directed toward the outside. This last conception seems probably for the present to be the most acceptable, nevertheless, the fact that the pure solvent enters through the unmovable semipermeable membrane (as in PFEFFER'S experiments) is difficult of reconciliation with this mode of explanation. Obviously, and for physiological purposes, it seems best to make use of the former explanation, in which the osmotic pressure is considered as a measure of the force with which a solution attracts the solvent.

PFEFFER'S above-described method of directly determining the pressure can only be used in exceptional cases, first because the preparation of the semipermeable membrane is connected with difficulties, and second, because there are only a few crystalline bodies for which impermeable membranes have been found. There are other quicker and easier ways of determining the osmotic pressure.

Solutions of non-volatile substances boil at a higher temperature than the pure solvent. This is due to the fact that the dissolved substance, because of the osmotic pressure, holds on to the solvent with a certain force. As in boiling a part of the solvent is separated from the dissolved body, and as the osmotic pressure can be considered as a measure of the attractive power between the solvent and the dissolved substance, then it is clear that solutions which are prepared with the same solvent and have the same osmotic pressure (isosmotic solutions)

[1] Amer. Chem. Journ., **37**, 425, 558 (1907); **41**, 1, 257 (1909).

must also boil at the same temperature. The rise in the boiling-point of a solution above the boiling-point of the solvent (elevation of the boiling-point) is also, like the osmotic pressure, for dilute solutions proportional to the concentration.

Solutions have a lower freezing-point than the pure solvent, and as in dilute solutions the solvent can be frozen out from the dissolved body, then isosmotic solutions have the same freezing-point. The depression of the freezing-point is also proportional to the concentration.

The determination of the elevation of the boiling-point for the estimation of the osmotic pressure of animal fluids is applicable only in exceptional cases, because on heating, precipitates often form. The determination of the depression of the freezing-point has been found of much greater use. This can be accomplished in an easy manner by aid of the apparatus suggested by BECKMANN. In regard to the use of this method we must refer to more complete works.[1]

The above rule that equimolecular solutions of different bodies have the same osmotic pressure is only applicable to non-electrolytes. The electrolytes (bases, acids, salts) show in aqueous solution a much greater pressure (i.e., a much lower depression of the freezing-point) than equimolecular solutions of non-electrolytes. As is known, ARRHENIUS has explained this lack of correspondence by the assumption that the molecule of the electrolyte is divided or dissociated into so-called ions having an opposed electric charge. An ion exerts upon the osmotic pressure the same influence as the non-dissociated molecule. The larger the number of dissociated molecules the more does the osmotic pressure of the solution rise above the pressure of an equimolecular solution of a non-dissociated body. The osmotic action of a dissociated body is equal to that of a non-dissociated body which in a given volume contains as many molecules as the dissociated body contains ions plus non-dissociated molecules. If we assume that a is the degree of dissociation, i.e., the number of the molecules that are dissociated, then $1-a$ is the number that is not dissociated. If in the dissociation of a molecule n ions are formed then the relation of the molecules present before the dissociation to the ions + molecules present after the dissociation is $1:(1-a+na)$ or $=1:(1+[n-1]a)$. The expression $(1+[n-1]a)$ is generally denoted by the letter i, and can be directly determined by estimating the freezing-point of a solution of known molecular concentration.

A gram-molecule aqueous solution (one that contains as many grams per liter as the molecular weight of the substance) of any non-electrolyte freezes at about $-1.86°$, or, the depression of the freezing-point Δ is $=1.86°$. For example,

[1] Ostwald-Luther, Hand- und Hilfsbuch zur Ausführung physik.-chemischer Messung, 3 Aufl., 1910.

if we find that Δ for a gram molecular solution of NaCl is 3.40° then we have according to the above $1 : (1+[n-1]a) = 1.86 : 3.40$. In the dissociation of NaCl two ions are formed, therefore $n=2$, and from the above equation the degree of dissociation can be calculated, $a=0.83$. The degree of dissociation can also be calculated from the electrical conductivity. Only the ions take part in the conduction of electricity, and the molecular conductivity $\left(=\frac{\text{conductivity}}{\text{molecular concentration}}\right)$ is proportional to the degree of dissociation. The dissociation increases with the dilution and at infinite dilution all molecules are dissociated ($a=1$). If we designate with $\mu\infty$ the limit value which the molecular conductivity approaches in infinite dilution and with μ_v the molecular conductivity at some definite dilution v, then the degree of dissociation at this dilution is $a=\frac{\mu_v}{\mu\infty}$.

The positively charged ions are called cations, and the negatively charged ones anions. Common for all acids are the positively charged H-ions while the negatively charged OH-ions are common for all bases.

Osmotic Experiments with Plant Cells. We often meet the word *osmosis* in literature without understanding exactly what is meant thereby. As a rule *diffusion streams* are meant, which are modified by means of the permeability conditions of an inclosing membrane. We now know that the driving force, namely, the streaming, is brought about by the differences in concentration, i.e., by difference in the osmotic pressure on the two sides of the membrane.

After NÄGELI found that certain plant cells, when they were treated with a sufficiently concentrated solution of certain substances, changed their appearance so that the protoplasm retracted,[1] DE VRIES studied this phenomenon further.[2] He called it *plasmolysis*. The most important substances for bringing about plasmolysis are the salts of the alkalies and alkaline earths, varieties of sugars, polyatomic alcohols, and neutral amino-acids. An indispensable condition for bringing about plasmolysis is that the solution must not have any destructive action upon the cells. NÄGELI gave the correct interpretation of plasmolysis, which is that those bodies which plasmolyze plant cells pass through the cell membrane of the cell, but not through the protoplasmic layer which follows. Instead of this the substance attracts water from the inner parts of the cell. The cell contents surrounded by protoplasm therefore diminish in volume and the protoplasm recedes more or less from the cell membrane. From this it follows that only those solutions whose power of attracting water is greater than that of the cell contents can bring about plasmolysis. As the ability to attract water (or the osmotic pressure) increases with concentration, there must be a limit solution for every substance above which all higher concentrations plasmolyze. The limit solution is called *isotonic* with the cells;

[1] Pflanzenphysiol. Untersuch., 1855.

[2] Eine Analyse der Turgorkraft, Jahresber. f. Wissensch. Botanik, 14, 427 (1884).

weaker solutions are called *hypotonic*, and stronger *hypertonic*. DE VRIES, with the aid of equal cells (cells of the epidermis of the lower side of the leaf of the Tradescantia discolor) has, for various substances, determined the concentration of this limit solution. It was found that the limit solution of analogously constructed salts had the same molecular concentration. Thus the alkali salts of the type NaCl (haloid salts, nitrate, acetate) plasmolyzed at one molecular concentration and the salts of the type Na_2SO_4 (sulphate, oxalate, diphosphate, tartrate) at another concentration. If the plasmolyzing power of a molecule of the first group is equal to 3, then the molecule of the second group equals 4. The concentration of the limit solution varied in DE VRIES' experiments between the limits corresponding to a NaCl solution of 0.6–1.3 per cent.

As above mentioned, only those substances bring about plasmolysis which cannot themselves pass through the protoplasm envelope of the cell content, and these substances only in the case that the concentration is sufficient. If a body is taken up by the protoplasm it produces no plasmolysis, because its tendency to attract water has been satisfied by its own passage into the cell. These substances do not produce plasmolysis in any concentration. If a body slowly passes in, then at first it causes plasmolysis, but this then ceases later. The plasmolytic methods have been used by DE VRIES, and especially by OVERTON.[1]

Experiments with Blood Corpuscles. Over a hundred years ago HEWSON observed that the blood corpuscles were destroyed in water, and that salts in certain concentrations prevented destruction.[2] HAMBURGER[3] has carefully and systematically investigated the action of salts of the alkalies and alkaline earths, and concludes that when blood is mixed with certain volumes of solutions of different concentrations of the same salt, all solutions whose concentration lie below a certain limit cause the exudation of hæmoglobin. On comparing the molecular concentration of the limit solution of different salts it was found that they bore the same relation to each other as the relative figures found by DE VRIES for the molecular concentration of the plasmolytic salt solutions. From this it probably follows that the protective action of the salts upon the blood corpuscles depends upon the same reason as the plasmolysis. This conclusion is also supported by the fact that those substances which, according to DE VRIES, in proper concentration cause plasmolysis in living plant cells, can also under similar conditions prevent the exudation of hæmoglobin. Those bodies, on the contrary,

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[1] Vierteljahrschr. d. Naturf. Gesellsch. zu Zurich, **40**, 1 (1895); **41**, 383 (1896).

[2] Phil. Trans., 1773, p. 303.

[3] Arch. f. (Anat. u.) Physiol., 1888, p. 31; Zeitschr. f. Biol., **26**, 414, (1889).

which do not cause plasmolysis, act in aqueous solution in the same manner upon the blood corpuscles as pure water. This has been especially shown by the investigations of GRYNS.[1]

Different investigators have attempted to perform plasmolytic experiments with animal cells, but without any special result. With the microscope one can often observe that the red blood corpuscles shrink under the influence of a strong salt solution, but the limit solution when the shrinking just begins cannot be exactly determined because the changes in volume are so very small. If we summate the changes in volume of many corpuscles, which can be done by centrifuging the blood mixture in a graduated tube, then very small changes can be detected. Such determinations have been made by HEDIN,[2] KOEPPE [3] and others. It was found that the blood corpuscles swell in a weak salt solution, shrink in a stronger solution, and there is a certain concentration which does not change the volume. By determining the freezing-point HEDIN found that this concentration for NaCl was nearly isosmotic with the serum of the blood corpuscles used. The depression of the freezing-point was about 0.56° and the concentration of the NaCl solution is 0.9 per cent, or about 0.15 normal.

The question as to the permeability of the blood corpuscles has been investigated by HEDIN, using a method depending upon the following:[4]

The depression of the freezing-point of a solution is proportional to its concentration. A certain amount of the substance to be tested is dissolved in blood. The serum of this treated blood freezes at a lower temperature than before the salt was added. The depression of the freezing-point can be designated as a. Now the same amount of substance is dissolved in serum using the same volume of serum as blood was previously used. The depression of the freezing-point of this serum can be designated as b. From this it is evident that $a = b$ if the blood corpuscles take up just as much dissolved substance from the blood as an equal volume of serum. If the blood corpuscles take up less than the serum then $a > b$ or $\frac{a}{b} > 1$, and when they take up more than the serum then $a < b$ or $\frac{a}{b} < 1$.

The result $\frac{a}{b}$, in the calculation of which the change taking place in the volume of the blood corpuscles on the addition of the substance must be considered, gives immediately an approximate idea of the quantity of substance which has passed into the blood corpuscles.

The results were as follows:

The salts of the fixed alkalies and alkaline earths, neutral amino-acids, varieties of sugars as well as hexatomic and pentatomic alcohols pass into the blood corpuscles only to a slight degree. Erythrite (tetra-

[1] Pflüger's Arch., **68**, 86 (1896).
[2] Skand. Arch. f. Physiol., **5**, 207, 238, 377 (1895).
[3] Arch. f. (Anat. u.) Physiol., 1895, 154.
[4] Pflüger's Arch., **68**, 229 (1897); **70**, 525 (1898).

tomic alcohol), passes slowly, and glycerin (triatomic) also passes slowly, but faster than erythrite. Ethylene glycol (diatomic alcohol) passes rather rapidly, and the monatomic alcohols immediately divide themselves equally in the serum and blood corpuscles. Ether, esters, aldehyde, and acetone divide themselves so that the blood corpuscles contain more than does an equal volume of serum. These bodies are equally absorbed by the blood corpuscles. Ammonium salts with univalent anions pass in quickly while with divalent or polyvalent anions the greater part remains in the serum; still they pass in to a greater extent than do the corresponding salts of the fixed alkalies.

OVERTON had previously arrived at the same results, using plant cells and chiefly by making use of the plasmolytic method. Urea is probably more quickly taken up by the blood corpuscles than by plant cells, and ammonium salts also seem to pass more easily into the blood corpuscles than into the plant cells.

In regard to other salts HEDIN'S results have been substantiated by OKER-BLOM,[1] by estimating the electrical conductivity of the blood.

It must also be stated that according to HEDIN, only those bodies which do not pass, or pass slowly into the cells, can essentially alter the volume of the cells. A close correspondence exists in this regard between the plant and animal cells.

GÜRBER found that when blood corpuscles are repeatedly washed with salt solution until the wash solution does not show any alkaline reaction, and are then suspended in NaCl solution and treated with CO_2, the alkaline reaction increased while the blood corpuscles became richer in chlorine. No exchange of K or Na took place.[2] GÜRBER explains the experiment as follows: the carbonic acid set a small amount of HCl free from the salt, and this HCl was taken up by the blood corpuscles. The Na_2CO_3 formed at the same time gave the alkaline reaction to the solution. KOEPPE[3] as well as HAMBURGER and v. LIER[4] claim, on the contrary, that an exchange of HCO_3-ions and Cl-ions takes place between the blood corpuscles and the solution, and HAMBURGER and v. LIER claim to have shown that the blood corpuscles are permeable only for anions, while the cations do not pass in.

HAMBURGER[5] and his collaborators have also found about the same osmotic phenomena with other free mobile cells such as leucocytes, spermatozoa as with the red blood corpuscles. The osmotic relations have also been tried with intact parts of organs, therefore with cells

[1] Pflüger's Arch., 81, 167 (1900).
[2] Sitzungsber. d. med. phys. Gesellsch. zu Würzburg, 1895.
[3] Pflüger's Arch., 67, 189 (1897).
[4] Arch. f. (Anat. u.) Physiol., 1902, 492.
[5] Osmotischer Druck und Ionenlehre, Wiesbaden, 1902, 1, 401.

in connection with other tissue constituents. By investigations on the changes in the weight (instead of the volume changes in the above-mentioned experiments with plant cells and blood corpuscles) which frog muscles undergo in solutions, various experimenters, NASSE,[1] LOEB,[2] and OVERTON,[3] have tried to prove the ability of muscle to take up various substances. OVERTON found that as long as the irritability of the muscle was retained the muscle took up the same bodies as the plant cells. The sarcolemma is not responsible for the permeability, but the outer layers of the muscle protoplasm are.

The skin of amphibians seems according to OVERTON to behave like the muscles[4] in regard to permeability.

Theories of Admissibility. On what does the permeability or non-permeability of membranes and of cells for certain bodies depend? The discoverer of precipitation membranes, M. TRAUBE, considered the membrane as a sort of molecular sieve. The relation of the size of the particles passing and the width of the pores of the membrane is important.[5] This view cannot be contested. The copper ferrocyanide membrane may be considered to act in this way and the non-permeability of most membranes for colloid substances depends upon the fact that the pores are too narrow for the particles.

The question as to the occurrence of a special outer limiting layer of the cells is of interest for the understanding of the metabolism of the cells as well as for the knowledge as to the manner in which the cells take up and give out substances. In this connection it must be recalled that in the protoplasm of certain cells we find an outer dense layer or a true membrane which seems to consist of protein substances. Still, even in cells in which no special outer limiting layer can be seen, the presence of such a limiting layer must be admitted because of the permeability conditions of these cells.

NERNST[6] has shown, by special experiments, that the permeability of a membrane for a certain substance is essentially dependent upon the solvent power of the membrane for this substance. This question which is very important for the study of the osmotic phenomenon in living cells has been especially studied by OVERTON.[7] From the behavior

[1] Pflüger's Arch., **2**, 114 (1869).
[2] *Ibid.*, **69**, 1; **71**, 457 (1898).
[3] *Ibid.*, **92**, 115 (1902); **105**, **176** (1904).
[4] Verhandl. d. phys. med. Gesellsch. zu Würzburg (N. F.), **36**, 277 (1904).
[5] Arch. f. Anat. Physiol. u. Med., 1867, 87.
[6] Zeitschr. f. physikal. Chem., **6**, 37 (1890).
[7] Vierteljahrsschr. d. Naturf. Gesellsch. in Zurich, **44** (1899) and Overton, Studien über die Narkose, Jena, 1901.

of living cells to dye-stuffs, as well as the special ease in which certain substances, which are not soluble in water or only slightly so, but are readily soluble in fats or fat-like bodies, pass into animal and plant protoplasms has led OVERTON to the conclusion that the protoplasmic limiting layer behaves like a substance layer having the solvent properties similar to the fatty oils. According to OVERTON the protoplasmic layer is probably impregnated with *lipoids*, i.e., bodies more or less similar to the fats in regard to their solubilities and their solvent power upon certain substances. The lipoids do not form a chemically definable class of bodies. Certain of them are still of an unknown constitution while others are known, especially the lecithins (the phosphatides as a group) and the cholesterin are to be especially mentioned on account of their great importance.

The assumption that an accumulation of lipoids occurs, as a special limiting layer, in the cells is not sufficiently founded and not generally true at least for the animal cells. Still this assumption is not absolutely necessary for a comprehension of the action of lipoids in the above sense. Objections have been raised by a few investigators against OVERTON'S theory, which has found general acceptance.[1] Thus it fails to explain all cases, although this was suggested by OVERTON himself, for instance according to COHNHEIM, it does not explain the absorption processes in the intestinal canal, and according to MOORE and ROAF it cannot explain certain properties of the cells, namely the varied composition of the electrolytes within and outside of the cells, and the selective taking up of certain soluble substances such as food products, drugs, toxins and antitoxins by the cells. The investigations of the last mentioned experimenters are based essentially upon investigations of the behavior of mineral substances, and they show that the above theory offers certain difficulties in explaining the exceedingly important exchange of mineral substances between the cells and the external fluid. Also the fact that the cells are readily permeable for water is explained with difficulty by OVERTON'S theory.

J. TRAUBE[2] especially has put forth objections to OVERTON'S theory. According to him, the passage of a substance from a watery solution into the cells, is in the first place due to its so-called *solution tenacity* in the watery solution. This solution tenacity is according to TRAUBE the attraction between the solvent and the solute; and is not identical with the osmotic pressure, but is measured by the surface tension of the solution.

[1] See O. Cohnheim, Die Physiologie der Verdauung u. Ernährung (1908). J. Loeb in Oppenheimer's Handbuch der. Biochem. Bd. 2, 105. T. B. Robertson Journ. of biol. Chem., 4 (1908). B. Moore and H. Roaf, Biochem. Journ., 3 (1908).

[2] Pflüger's Archiv., **105**, 541 (1904); **122**, 419 (1908); **132**, 511 (1910); **140**, 109 (1911).

It has been shown that those substances which are not taken up by the cells at all or only slightly, do not lower the surface tension of the water when dissolved therein. On the contrary, those substances which lower the surface tension, pass into the cells. According to GIBBS those substances, which when dissolved in water lower the surface tension, occur in greater concentration on the surface as compared with the interior. Thus according to TRAUBE the solution tenacity is less the lower the surface tension of the watery solution. Otherwise the direction of movement of a substance in the boundary between two phases (watery solution and cells) is determined by the relationship between the solution tenacity of the substances in the two phases. However, the solution tenacity of a substance can only be directly measured in the watery solution. TRAUBE supports his theory upon different experiments in which members of the same homologous series were dissolved in water in such concentration as to have the same surface tension and also showed the same ability to pass into the cells. The disagreement in other cases can be explained by the unknown solution tenacity in the cell phase. As we will show below TRAUBE'S proposition calls to mind the accepted views as to the origin of the adsorption phenomena or the taking up of dissolved substances by solid bodies. LÖWE[1] has also found, in studying the taking up of different dissolved substances by lipoids, that the process does not take place as called for by OVERTON'S theory according to HENRY'S law of absorption but rather an adsorption.

Certain substances which are of the very greatest importance for life processes and which probably are burned to a great extent within the cells, have according to the above experiments only a limited ability to enter the cells. These bodies are the sugars and the amino-acids. Also the presence of salts within the cells is not easily understood in view of the above experiments. In consideration of this it must be remarked that the above described experiments on the permeability of animal cells have been carried out with cells that were removed from their attachment to the living animal. Although these cells are not considered as physiologically dead cells still it is very probable that certain life functions have been arrested. It is readily conceivable that the oxidation processes, whereby the organic substances taken up within the cells are transformed into simpler products, are at least partly brought to a standstill (see Chapter XVI). That, nevertheless, at least salts and sugar also attract water in the living organism and therefore only pass into the cells in small quantities follows from the experiments of HEIDENHAIN, according to whom these substances are designated as lymph forming agents of the second order (Chapter VI). This action is also explained

[1] Bioch. Zeitschr., 42; 150, 190, 205, 207 (1912).

by HEIDENHAIN as being dependent upon their power of abstracting water from the tissues.

If we admit that the cells normally contain only small amounts of sugar and amino-acids at any one time, then, if these substances are being continuously burned within the cells, new quantities must constantly be taken up and in this way gradually large quantities of the mentioned substance would be taken up and burned. If the combustion is arrested no new quantities are taken up. The fact that certain substances are only taken up in small quantities at a time does not prove that they are not burned within the cells.

According to MOORE and ROAF[1] the salts exist in the blood corpuscles in the form of "adsorpates;" these are adsorbed by the solid constituents of the blood corpuscles. As we will see further on (page 27) an adsorbing substance can only take up a limited amount of another substance. If, after the saturation limit is reached, more of the adsorbed substance is added then practically no more is taken up. In this way we can explain why the blood corpuscles only take up very little of the salts added. The slight ability of the sugars and amino-acids to be taken up can perhaps be explained in a similar manner.

Osmotic Pressure of Animal Fluids. As is apparent from the above, a substance exerts upon living cells an entirely different influence, depending upon whether the substance is able to pass into the cell or not, and whether the substance which does not pass in has the ability of attracting water or not. Therefore that part of the osmotic pressure of body fluids which is caused by bodies not passing in is called the *effective osmotic pressure.* In this manner therefore the salts of the alkalies and alkaline earths and the sugars act. As sugar, as well as the bodies which according to the just mentioned experiments are readily taken up by the cells, occurs under ordinary conditions only in very small amounts in the blood, and also as the proteins are practically without influence upon the osmotic pressure, the normal osmotic pressure of the blood is chiefly due to the salts. As the depression of the freezing-point is almost the only method used for animal fluids, therefore ordinarily the freezing-point depression (Δ) is given as a measure of the osmotic pressure. For mammalian blood Δ is constant with the exception of slight variations due to the food and perhaps also to other circumstances. It is 0.56°,[2] which corresponds to a 0.90 per cent NaCl solution and to an osmotic pressure of about 6½ atmospheres. In lower animals Δ may be slightly lower, for example, in the frog $\Delta = 0.46°$. In invertebrate sea animals the body fluid is equal to the osmotic pressure of the sur-

[1] Bioch. Journ., **3**, 55 (1908).

[2] Hamburger, Osmotischer Durck u. Ionenlehre, **1**, 456.

rounding sea water ($\Delta = 2.3°$) and varies with the quantity of salt in the water (BOTTAZZI). In lower fishes (Selachii) the osmotic pressure of the blood is equal to the surrounding medium, and in higher fishes (Teleostomi) lower ($\Delta = 1.0°$) (BOTTAZZI). In Selachii the osmotic pressure of the blood is chiefly due to urea (SCHROEDER).[1]

In sea fishes as well as fresh-water fishes, for example, the eel, a lower osmotic pressure ($\Delta = 0.41°$) is found when kept in fresh water than when kept in sea water ($\Delta = 0.55°$)[2]. In lower sea animals the osmotic pressure is equal to the surrounding medium, while higher animals are independent of the surroundings. HÖBER calls attention to this condition and points out the analogy with the body heat of the various animals.[3]

If we pass to other body fluids we must mention that the lymph shows a somewhat higher osmotic pressure than the blood, and this is due to the lymph taking up from the tissues metabolic products having a low molecular weight.[4] Milk and bile have the same osmotic pressure as the blood,[5] while saliva has a lower pressure.[6] The urine of man and mammalia generally has a much higher osmotic pressure than the corresponding blood.[7] For human urine Δ varies between 1.3 and 2.3°. After abundant drinking as well as under pathological conditions (diabetes insipidus) the osmotic pressure of the urine can be lower than the blood. In regard to the osmotic pressure of animal fluids under normal and pathological conditions we refer to the work of KORÁNYI and RICHTER.[8]

II. COLLOIDS.

The word *colloid* originated with GRAHAM, who included in this name different substances which did not have the property of diffusing through an animal membrane. In opposition to this GRAHAM called those bodies which passed through a membrane, *crystalloids*, because they were as a rule crystalline, a property which with few exceptions does not belong to the colloids.[9] GRAHAM included soluble silicic acid among

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[1] Bottazzi, Archives ital. de biol. **28**, 61 (1897). Schroeder, Zeitschr. f. physiol. Chem. **14**, 576 (1890).

[2] Dekhuisen, Arch. néerland, **10**, 121 (1905); Quinton, Compt. rend. soc. biol., **57**, 470, 513 (1904).

[3] Physik. Chem. d. Zelle u. Gewebe, **3**. Aufl. 353, (1911).

[4] Leathes, Journ. of Physiol., **19**, 1 (1895).

[5] Dresser, Arch. f. exp. Path. u. Pharm., **29**, 303 (1892).

[6] Nolf, Travaux du lab. de phys. de Liège, **6**, 225 (1901).

[7] Korányi, Zeitschr. f. klin. Med., **33**, 1 (1897), **34**, 1 (1898).

[8] Physikalische Chemie und Medizin. Leipzig (1907).

[9] Ann. d. Chem. u. Pharm., **121**, 1 (1862) as well as Ann. de chim. et de Phys. (4), **3**, 127 (1864).

the colloids and also analogous forms of stannic acid, titanic acid, molybdic acid and tungstic acid, aluminium hydroxide and analogous metallic oxides, when they exist in the soluble form, and also starch, dextrins, the gums, caramel, tannin, albumin and gelatin.

Some colloids are characterized by the fact that under certain conditions they solidify into a gelatinous form containing considerable water. In the case where water is the solvent then GRAHAM called the soluble form *hydrosol* and the gelatinous form *hydrogel*.

By diffusion through a membrane (called *dialysis* by GRAHAM) colloid substances can be separated from crystalloids. Colloidal silicic acid as well as corresponding forms of certain other bodies are obtained by treating the soluble alkali salt with hydrochloric acid, then removing the excess of hydrochloric acid as well as of chlorides, by means of dialysis. Colloidal alumina was obtained by GRAHAM by dissolving aluminium hydroxide in aluminium chloride. This last salt was removed by dialysis and the hydroxide remained with more or less HCl combined in solution.

Various metallic sulphides can be obtained in colloidal solution. Such solutions of As_2S_3 and Sb_2S_3 can be obtained by passing H_2S into dilute solutions of the respective metallic oxide,[1] and colloidal CuS can be prepared by washing the precipitated compound with water, by which treatment the CuS finally becomes soluble in water.[2]

The metals can be obtained as hydrosols, and indeed in two ways:

1. By treating a salt with various reducing agents (for example formaldehyde, hydrosulphurous acid, hydrazine, hydroxylamine) the various metals are obtained in colloidal solution.[3] As the solutions thus obtained are often very unstable, it has been found advisable to help their stability by the addition of organic colloids (gelatin). We will discuss the mode of action of these so-called protective colloids on page 23.

2. BREDIG [4] has discovered a method which makes possible the production of pure metallic sols by the cathode spraying of metallic wires under water. SVEDBERG [5] prevents the heating of the fluid in this spraying by using the induction current. This makes the spraying also possible under organic fluids and sols of the light metals have also been prepared. Practically sols of all metals and metalloids can be prepared in this way.

Among those bodies which can be obtained in the colloidal state we have acids as well as bases, and the chemical elements are also known as colloids, as well as bodies of more complex molecular structure like the proteins and starches. The colloid bodies, therefore, have from a chemical standpoint nothing in common. More likely the colloidal condition is due to physical properties, and this follows from the researches of GRAHAM. The crystalloids and the colloids are therefore not to be considered as chemically different classes of bodies, but rather only as different physical conditions of matter and the boundary between

[1] H. Schulze, Journ. prakt. Chem. (N.F.), **25**, 431 (1882), and **27**, 320 (1883).
[2] Spring, Ber. d. d. chem. Gesellsch., **16**, 1142 (1883).
[3] Müller, Allg. Chemie d. Kolloide. Leipzig (1907), 6.
[4] Anorganische Fermente. Leipzig (1901), 24.
[5] Ber. d. d. chem. Gesellsch., **38**, 3616 (1905); **39**, 1705 (1906).

these two conditions is often very indefinite. Certain chemically definable classes of substances, such as proteins, occur only or chiefly in the colloidal condition while others, such as the inorganic salts, occur as crystalloids. Finally we find others that can occur in both forms, namely the soaps (page 17). In short the difference between the crystalloid and colloidal condition may be considered in that the crystalloids occur in solution as molecules of medium size while the colloids are either very large molecules, molecular aggregations or at least particles of a larger spacial volume than the crystalloids. According to such a conception many properties of the colloids can be explained.

In order to give a better review we will give a classification of the colloids which seems, for the present, to be rather universally accepted. This was first suggested by PERRIN[1] and later accepted by HÖBER,[2] A. MÜLLER,[3] and WO. OSTWALD,[4] although different authors use different names for the two classes. The classifications of HARDY[5] and ZSIGMONDY[6] have also much in common with the classification given below.

One of the two groups of colloids is called *hydrophile colloids* (emulsion colloids, emulsoides) because in the aqueous solution a certain relation still exists between the dissolved substance and the solvent which is evident especially by a certain viscosity of the solution. The hydrophile colloids often gelatinize on cooling, the gel is again soluble in water (reversible), and in general the hydrophile colloids are separated from their solution by electrolytes with greater difficulty than the colloids of the second group. Bodies of the greatest importance for physiological chemistry like the proteins, starch, glycogen, and soaps in watery solution belong to the hydrophile colloids.

Contrary to the hydrophile colloids, the colloids of the colloidal metal type are called *suspension colloids* (suspensoids) as they must be considered as suspended solid particles in a solvent and have no close relation to the solvent. The viscosity of the solution does not differ much from that of the pure solvent; besides this, the suspension colloids do not gelatinize, do not swell up, and are readily precipitated by electrolytes. To this group belong the metallic sols, the colloidal metallic sulphides, and certain typical suspensions obtained by dissolving water-insoluble substances in another liquid (alcohol, acetone) and then pouring this solution into a large volume of water. In this way the substance is precipitated in a finely divided condition. Such suspensions

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[1] Journ. de Chimie phy., **3**, 84 (1905).
[2] Physik. Chem. d. Zelle u. Gewebe, 2 Aufl. (1906), 208.
[3] Allg. Chemie d. Kolloide (1907), 187.
[4] Zeitschr. f. Chem. u. Ind. d. Koll., 1, 331 (1907).
[5] Proc. Roy. Soc., **66**, 95 (1899).
[6] Zur Erkenntnis d. Koll. (1905), 16.

behave in many respects like suspension colloids. Suspensions of mastic,[1] colophony,[2] and cholesterin[3] belong to this class.

The hydrophile colloids stand closer to the crystalloids than do the suspension colloids, and the transition between the crystalloids and the hydrophile colloids is only gradual. At the boundary we find the peptones and proteoses which belong to the proteins, but at the same time dialyze rather well. On the other hand, we also have colloids which to a certain extent form intermediary steps between the hydrophile colloids and suspension colloids. Finally, there are also numerous intermediary members between the suspension colloids and the finely divided substances suspended in water (kaolin).

Osmotic Pressure. As above stated, the osmotic pressure of solutions of crystalloids can be determined only in exceptional cases by means of the semipermeable membrane, because it is very difficult to prepare membranes which are impermeable for crystalloids. As previously stated, most membranes are impermeable for colloids, and the osmotic pressure of the colloids can be best directly determined by the aid of a membrane in a so-called *osmometer*. As shown by MOORE and ROAF, in such an apparatus changes in pressure can be determined which are not detectable by the determination of the freezing-point.[4]

Equimolecular solutions of various non-electrolytes give the same osmotic pressure. From this it follows that when different non-electrolytes exist in solutions with the same percentage concentration, the osmotic tension of these solutions must be in inverse proportion to their molecular weights. Certain colloids which will be discussed in another connection (proteins, glycogen, etc.) must have a very large molecule. From this it follows that these bodies must exert a very low osmotic pressure. The proteins always contain a small amount of salts which exist either in a sort of combination with the colloids or are to be considered as contaminations which are difficult to remove. For this reason it has been repeatedly stated that these salts are responsible for the small differences in the osmotic pressure. By carefully washing crystalline proteins from serum and egg-white, REID was able to prepare bodies which gave finally no osmotic pressure in the osmometer.[5] In opposition to this, MOORE and ROAF as well as LILLIE call attention to the fact that the osmotic pressure of protein solutions is influenced by the treatment which the protein received before the determination.

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[1] Zeitschr. f. physik. Chem., **57**, 47 (1906).
[2] *Ibid.*, **38**, 385 (1901).
[3] Bioch. Zeitschr., **7**, 152 (1908).
[4] Bioch. Journ., **2**, 34 (1906).
[5] Journ. of Physiol., **31**, 438 (1904).

STARLING,[1] MOORE and PARKER,[2] MOORE and ROAF[3] and LILLIE,[4] using protein preparations which had not been exposed to any strong treatment before use (serum proteins, ovalbumin), as well as REID[5] (with hæmoglobin), have been able to detect a low osmotic pressure and indeed by the aid of osmometric methods. According to STARLING, the proteins of the serum correspond to a pressure of 30–40 mm. Hg. and REID[6] found a pressure of 3–4 mm. Hg. for a 1 per cent hæmoglobin solution.

The influence of added bodies upon the osmotic pressure has been tested by LILLIE by adding the substance to be tested in the same percentage concentration to the inner and outer fluids. It was found that non-electrolytes were without action while acid and alkalies increased the osmotic pressure of gelatin solutions, while salts lowered the pressure of gelatin as well as ovalbumin solutions. ADAMSON and ROAF[7] arrived at similar results in regard to alkalies and acids. Besides this, LILLIE found that the osmotic pressure was dependent upon the past history of the colloid. Warming as well as shaking the solutions seems to change the aggregate condition, which returns very slowly or not at all. The changes in the osmotic pressure produced by salts, LILLIE explains by a change in the aggregate condition of the colloid, by the addition of salts it is brought closer to its precipitation point and is probably united in large aggregations. In this way the number of particles is diminished and, as this number must be important for the osmotic pressure, this pressure is lowered. In agreement with this the above mentioned influence of acids and alkalies upon the osmotic pressure of gelatin can be explained by an increase in the particles.[8]

As we have seen above the determination of the elevation of the boil ing-point or the depression of the freezing-point is the simplest way for estimating the osmotic pressure of a crystalloid substance in solution. If such determinations are made with a colloidal solution then unmeasurable results are found for the elevation of the boiling-point or the depression of the freezing-point. This indicates, as above stated, that the molecules or the particles must be very large. F. KRAFT[9] found no elevation of the boiling-point for soaps in watery solution but obtained values which correspond to the calculated molecular weights when the soaps were dissolved in alcohol. Therefore the soaps are colloidal in watery solution and crystalloidal bodies in alcoholic solution.

Filterability. Large particles suspended in a liquid can be removed from the fluid by filtering. The finer the suspended particles are the

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[1] Journ. of Physiol., **19**, 322 (1896).
[2] Amer. Journ. of Physiol., **7**, 261 (1902).
[3] Bioch. Journ., **2**, 34 (1906).
[4] Amer. Journ. of Physiol., **20**, 127 (1907).
[5] Journ. of Physiol., **33**, 12 (1905).
[6] Bioch. Journ., **3**, 422 (1908).
[7] *Ibid.*
[8] Pauli, Koll. Zeitschr., **7**, 241 (1900).
[9] Ber. d. d. chem. Gesellsch., **29**, 1328 (1896); **32**, 1584 (1899).

closer must the filter be. Extensive experiments on the filtering of colloids have been carried out by BECHHOLD.[1] He used paper filters which were impregnated with collodion dissolved in glacial acetic acid. According to the concentration of the collodion solution filters of different porosity were obtained. The colloid solutions were pressed through the filter by a pressure up to five atmospheres. It was shown that all colloid solutions contained particles of various sizes. Nevertheless for every solution a filter could be prepared whose pores were small enough to retain all the particles. In this manner BECHHOLD was able to classify the colloids in a series according to the size of the smallest particles. He found that in general the inorganic colloids (Prussian blue, platinum, iron oxide, gold, silver) form larger particles than the organic colloids (gelatin, hæmoglobin, seralbumin, proteoses, dextrin). Still it must be remarked that according to ZSIGMONDY[2] the size of the particles of the same colloid are larger in one preparation than in another and that the size can change on keeping.

On filtering proteose solutions through filters of unequal thickness BECHHOLD was able to show that the larger the particles of the proteoses, the easier are they precipitable by ammonium sulphate.

Diffusion. We have already seen that the osmotic pressure of a colloid solution is very small and also that the osmotic pressure of a solution is the cause for the diffusion of the particles, therefore it is evident that the diffusion ability of colloids can only be very slight. This is not only true for the free diffusion but also for the diffusion through a membrane. Both of these was first studied by GRAHAM. The first was found very slight but measurable in several cases while the fact that the colloids did not diffuse through membranes (non-dialysable) was given as the most constant difference between colloids and crystalloids. Nevertheless, there does not exist any sharp boundary and dialysis depends principally upon the size of the particles as well as upon the character of the membrane.

Internal Friction. By the internal friction of a fluid we mean the force which resists the displacement of the particles of the fluid among one another. The internal friction is therefore an expression for the great thickness or viscosity of the fluid.

For physiological purposes the internal friction is determined by measuring the time which a given volume of the fluid requires to flow through a capillary tube under a pressure of its own weight.

It is generally accepted that the internal friction of suspension col-

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[1] Zeitschr. f. physik. Chem., **60**, 257 (1907).

[2] Zur Erkenntnis d. Koll., (1905), 104 as well as Zeitschr. f. Elektrochem., **12**, 631 (1906).

loids is equal to that of the pure solvent or differs from it only slightly. On the contrary hydrophile colloids are, in proper concentration, very viscous which is probably the reason that they gelatinize under certain circumstances. PAULI as well as PAULI and HANDOVSKY[1] have investigated strongly dialyzed serum in regard to its internal friction. The addition of a little salt (to 0.05 normal) causes a lowering of the internal friction below that of a pure albumin solution, while acids and alkalies in small amounts cause a powerful rise in the viscosity.

Optical Properties. Colloidal solutions are opalescent by reflected light, which depends upon the fact that the light is reflected by the suspended particles. The reflected light is partly polarized. This phenomenon, called TYNDALL'S phenomenon, depends upon the presence of small particles in the liquid, and is considered as a test for colloid solutions. Still there are colloid solutions (certain gold solutions, ZSIGMONDY), which do not give TYNDALL'S phenomenon, and on the other hand we also have solutions of certain high molecular crystalloids (cane sugar, raffinose), which produce this phenomenon.[2]

With the aid of the ultramicroscope of SIEDENTOPF and ZSIGMONDY, it has been made possible to see the colloidal particles directly.[3] In this apparatus the colloidal particles are strongly illuminated by direct light, so that no ray of light falls directly into the eye of the observer. The particles are hereby made visible on account of the formation of diffraction disks which are visible through the miscroscope. In colloidal solutions where the particles are close together, a more or less intense, homogeneous, polarized sphere of light is seen in the microscope where the individual particles cannot be distinguished from each other. This is possible on diluting the solution. Those particles which are only made visible by dilution are called *submicrons*, while those that gradually disappear on dilution are called *amicrons*.

The investigations of ZSIGMONDY and others upon the growth of colloidal metallic particles are also interesting. Thus the reduction of gold chloride by formaldehyde, whereby colloidal gold is formed, is accelerated by the addition of colloidal gold, and the added particles indeed grow at the cost of the newly reduced gold.[4] In a similar manner the reduction of silver nitrate with ammonia and formaldehyde is helped by the addition of colloidal gold when the reduced silver precipitates upon the gold particles.[5] In such processes the amicrons can enlarge so that they can be observed by the ultramicroscope (submicrons).

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[1] Pauli, Koll. Zeitschr., **3**, 5 (1908); Pauli and Handovsky, Biochem. Zeitschr., **18**, 340 (1909); **24**, 239 (1910).

[2] Lobry de Bruyn and Wolff, Rec. trav. chim. des Pays-Bas., **23**, 155 (1904).

[3] Zsigmondy, Colloids and the Ultramicroscope, translated by Alexander, New York, 1909.

[4] Zsigmondy, Zeitschr. f. physik. Chem., **56**, 65 (1906).

[5] Zsigmondy and Lottermoser, *ibid.*, **56**, 77 (1906).

According to the manner of preparation the colloids may have particles of different sizes. (See page 00.)

Submicrons have also been detected in solutions of organic colloids. The work of GATIN-GRUZEWSKA and BILTZ,[1] who used a specially pure glycogen, must be especially mentioned. They found that the aqueous solution of glycogen contained amicrons as well as easily recognizable submicrons, whose presence was only evident by a homogeneous sphere of light, but on the addition of alcohol, conglomerate into detectable submicrons.

Molecular Movement. R. BROWN[2] first found that small particles suspended in water showed a quivering motion, and this phenomenon has been called, from its discoverer, Brownian molecular motion, although the particles in no manner are to be considered as molecules. This phenomenon has been observed since then by many investigators in fluids having suspended solid particles as well as in substances dissolved in colloidal condition.

The Brownian movement is considered by some as a manifestation of a general molecular movement of matter. According to this view it is comparable with the supposed motion of gas molecules according to the kinetic theory of gases. PERRIN as well as SVEDBERG[3] claim that the law of gases also holds for very dilute colloidal solutions.

Electrical Transportation of Suspended Particles. A not too weak electric current has the power of causing motion in small quantities of fluid enclosed in a capillary tube or in a porous diaphragm. The particles suspended in a fluid also wander under the influence of the electric current, and indeed to the anode or cathode, according to the nature of the fluid and the particles. This phenomenon is called *cataphoresis*. Such movements have also been found in colloidal solutions. According to BILTZ,[4] in dialyzed aqueous solution, the colloidal metallic hydroxides wander to the cathode, and the other colloids (metals, metallic sulphides, acids) wander to the anode. The colloidal particles in water are therefore probably electrically charged, hence the negatively charged wander to the anode and the positively charged to the cathode. Dialyzed protein solutions show according to older investigations no cataphoresis. The addition of acid or alkali gives to the protein a positive or negative charge respectively, hence an alkaline solution wanders to the anode and an acid solution to the cathode (HARDY,[5] PAULI[6]). According to MICHAELIS[7] the proteins in perfectly neutral

[1] Pflüger's Arch., **105**, 115 (1904).

[2] Edinb. Phil. Journ., **5**, 358 (1828); **8**, 41 (1830).

[3] Perrin, Colloid-chem. Beihefte, **1**, 221 (1910). Svedberg, Koll. Zeitschr., **7**, 1 (1910).

[4] Ber d. d. chem. Gesellsch., **37**, 1095 (1904).

[5] Journ. of Physiol., **24**, 288 (1899).

[6] Hofmeister's Beiträge, **7**, 531 (1906).

[7] Biochem. Zeitschr., **16**, 81 (1909); **19**, 181 (1909); **24**, 79; **27**, 38; **28**, 193; **29**, 439 (1910); **32**, 456 (1911); **41**, 373 (1912).

solution wander to the anode in the case when the experiment is so carried out that the formation of acid or alkali is prevented at the anode or cathode respectively. If the neutral protein solution is treated with a trace of acetic acid then the particles wander to the cathode. With a certain very slight degree of acidity the direction of the wandering of the particles is reversed. With this reaction no wandering or a double-sided wandering of the protein bodies can be detected. This so-called *isoelectric point* has been determined by MICHAELIS, RONA and their collaborators for different protein substances.[1] MICHAELIS and RONA claim to have found in the isoelectric point the most favorable reaction for the heat coagulation of the protein substances, while SÖRENSEN and JÜRGENSEN consider the reaction which the pure protein substance gives to pure water as the optimal precipitation reaction.[2]

According to GATIN-GRUŽEWSKA[3] pure glycogen wanders distinctly to the anode.

Precipitation of the Colloids.

The colloids can be separated from their solutions in various ways. Many colloidal solutions are so unstable that they flock out after a time without the addition of anything (silicic acid, metallic hydroxides). Certain colloids appear as flocculent precipitates on heating their solutions (certain proteins, see Chapter II). Others solidify on cooling from hot concentrated solutions, as semisolid forms, so-called jellies or hydrogels, containing considerable water (glue, starch, agar).

On evaporating the hydrosols at ordinary temperature we obtain a residue which ZSIGMONDY divides into reversible and irreversible colloids, according whether they are again soluble in water or not.[4] According to this definition starch, dextrin, agar, gum, and proteins belong to the reversible colloids while colloidal silicic acid, stannic acid, colloidal metallic hydroxides and sulphides, and the pure colloidal metals belong to the irreversible colloids. The former are relatively non-sensitive toward the addition of electrolytes, while the latter flock out on the addition of the smallest quantity of electrolyte, and indeed again in an irreversible form. This classification stands in accord with what was given above (page 15), as the reversible colloids coincide in a measure with the hydrophile colloids and the irreversible with the suspension colloids.

Electrolyte Precipitation of Suspension Colloids. It must be remarked that for every precipitating electrolyte a certain minimal con-

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[1] See page 74.

[2] See Ergebnisse d. Physiologie, **12**, 506 which also gives the literature.

[3] Pflüger's Arch., **408**, 287 (1904).

[4] Zur Erkenntnis d. Koll., page 21.

centration is necessary to bring about flocking. In comparing the precipitation ability of various electrolytes the concentration of that solution which is just sufficient to cause a visible cloudiness is given in millimolls ($=\frac{1}{1000}$ gram-molecule) per liter.

HARDY[1] has also found that colloids which wander to the anode are chiefly flocked out by the cations of the precipitating electrolyte, and colloids wandering to the cathode are chiefly flocked out by the anions. H. SCHULTZE[2] has proven that the precipitating ability is influenced greatly by the valence of the precipitating ions, as the divalent ions act much stronger than the monovalent and the trivalent are still more active than the divalent. This rule has been substantiated by HARDY.[3]

This valence rule becomes clear by the following experiment of FREUNDLICH.[4] The figures give the lowest precipitation concentration expressed in millimolls per liter. The hydrosol was As_2S_3 (negative) and the valence of the cations is applicable chiefly for the precipitating action.

$\frac{K_2SO_4}{2}$	65.6	$MgCl_2$	0.717
KCl	49.5	$MgSO_4$	0.810
KNO_3	50.0	$CaCl_2$	0.649
NaCl	51.0	$SrCl_2$	0.635
LiCl	58.4	$BaCl_2$	0.691
$\frac{H_2SO_4}{2}$	30.1	$Ba(NO_3)_2$	0.687
		$ZnCl_2$	0.685
HCl	30.8	$UO_2(NO_3)_2$	0.642
		$AlCl_3$	0.0932
		$Al(NO_3)_3$	0.0982

The precipitating action of anions upon a positive hydrosol ($Fe[OH]_3$) is shown in the following experiment of FREUNDLICH:

KCl	9.03	K_2SO_4	0.204
KNO_3	11.90	Tl_2SO_4	0.219
NaCl	9.25	$MgSO_4$	0.217
$\frac{BaCl_2}{2}$	9.64	$K_2Cr_2O_7$	0.194

FREUNDLICH has extended the valence rule by the fact that with a negative sol, H ions, the ions of the heavy metals, as well as organic cations in weaker concentration, have a greater precipitating action than other cations; OH ions as well as organic anions act against the precipitating action of the cations. The reverse is shown with a positive sol; OH ions and organic anions of smaller precipitation concentration than corresponds to their valence; H ions and organic cations act against the precipitating properties of the anions.

Certain above-mentioned suspensions (mastic), as well as other particles suspended in water, act the same as suspension colloids. SCHULZE[5] has found that cloudiness due to clay particles on the addition of clarifying bodies (alum, lime) give a voluminous deposition. SCHLOESSING[6] found that clay suspensions

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[1] Zeitschr. f. physik. Chem., **33**, 385 (1900).
[2] Journ. prakt. Chem. (2), **25**, 431 (1882).
[3] Proc. Roy. Soc., **66**, 110 (1899).
[4] Zeitschr. f. Chem. u. Ind. d. Koll., **1**, 323 (1907).
[5] Ann. Phys. (2), **129**, 366 (1866).
[6] Compt. rend., **70**, 1345 (1870).

which do not settle after months are precipitated in 24–48 hours by a minimum quantity of lime or magnesia. He also calls attention to the essential rôle which the salts of sea water must play in the sedimentation of the cloudy fresh water flowing into the sea (delta formation).

In consideration of the conditions just mentioned, under which the suspension colloids are precipitated by electrolytes, the mutual precipitation ability of suspension colloids is of considerable interest. According to what has been stated previously, the colloids are considered as carriers of electricity, and it has been proved that the oppositely charged colloids can act precipitatingly upon each other. This rule was first proposed by LINDER and PICTON,[1] and has subsequently been substantiated by many investigators. BILTZ [2] has made especially systematic investigations on this subject and finds that colloids carrying the same kind of charge do not precipitate each other. For the mutual complete precipitation of opposed electrically charged colloids, a certain quantitative relation is necessary. On the action of two colloids with opposite charges in variable quantities an optimum of the precipitation action is noticed; while on overstepping the desirable precipitation conditions in both directions no precipitation occurs at all.

In analogy with the mutual precipitation ability of the colloids, BILTZ believes that the especial great ability of most salts of the heavy metals to precipitate colloids lies in the hydrolytically split and colloid-dissolving metallic hydroxides.

Protective Colloids. Certain hydrophile colloids, which are precipitated with difficulty by electrolytes, have the power of protecting suspension colloids against the precipitating action of electrolytes. MEYER and LOTTERMOSSER [3] have found with silver hydrosol that the presence of protein prevented the flocking out by electrolytes. ZSIGMONDY [4] has investigated the relative action of the protective colloids and has found considerable differences. The figure in milligrams of colloid which is just insufficient to protect 10 cc. of gold solution (0.0053–0.0058 per cent) against the action of 1 cc. 10 per cent NaCl solution is called the *gold equivalent* for the respective colloid. Gelatin offers the best protection, then comes isinglass, casein, ovalbumin, gum arabic, Irish moss, dextrin, starch. The colloidal sulphides (As_2S_3, Sb_2S_3, CdS) are also protected in the same manner against the influence of electrolytes (A. MULLER and ARTMANN [5]). Inorganic colloids may also act as protective

[1] Journ. chem. Soc., **71**, 572 (1897).
[2] Ber. d. d. chem. Gesellsch., **37**, 1095 (1904).
[3] Journ. prakt. Chem. (2), **56**, 241 (1897).
[4] Zeitschr. analyt. Chem., **40**, 697 (1901).
[5] Oester. Chem. Ztg., **7**, 149 (1904).

colloids. Thus according to BILTZ[1] zirconium hydroxide protects gold better than does gelatin.

By the addition of organic protective colloids, the inorganic colloids which on evaporation otherwise become irreversible, are made reversible, in that the dry residue is soluble in water again. On this depends the use of the protective action in the preparation of permanent inorganic hydrosols, and this is of importance in many cases.

According to BECHHOLD[2] the filterability of suspension colloids through collodion filters is increased by the addition of organic colloids. It is also well known that certain finely divided substances (carbon) pass more easily through a filter in the presence of protein than without protein.

The action of the protective colloids is ordinarily explained according to the theory of QUINCKE[3] on the mutual surface tension of the active bodies, and the process belongs accordingly to the adsorption phenomenon which will be discussed later. According to this theory the protective colloid under certain conditions spreads like an envelope around the particles. In this wise the entire mass takes the properties of the protective colloid and is therefore not precipitated by the electrolyte any more than the protective colloid itself. In filtration the protective colloid acts to a certain extent like a lubricant. This theory of colloid envelope has recently received support by experiments of MICHAELIS and PINCUSSOHN.[4] They found that when suspensions of indophenol and mastic were mixed together the number of particles visible in the ultramicroscope diminished; after mixing, the physical properties of the indophenol (pseudofluorescence, positive cataphoresis) were not evident.

Electrolyte Precipitation of Hydrophile Colloids. The salts of the alkalies precipitate the suspension colloids even in low concentrations. The alkali salts behave differently toward the hydrophile colloids. This may in part be due to the fact that hydrophile colloids have much less of a certain electric charge than the suspension colloids. For this reason the hydrophile colloids are often precipitated from their solution by alkali salts. For this purpose, firstly, certain concentrations are necessary; secondly, the precipitates of the hydrophile colloids are again soluble in water (reversible) in opposition to those of the suspension colloids. In regard to the ability of different alkali salts to act precipitatingly certain laws have been formulated, but they cannot be arranged in a general rule.

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[1] Ber. d. d. chem. Gesellsch., **35**, 4431 (1902).
[2] Zeitschr. f. physik. Chem., **60**, 301 (1907).
[3] Ann. Phys. (3), **35**, 580 (1888).
[4] Bioch. Zeitschr., **2**, 251 (1907).

On comparing the concentration of various salts just sufficient for precipitation, where at one time the same anion with different cations was tested and another time the same cation with different anions, PAULI has arranged the cations and anions in the following order in increasing precipitation ability:

$$CNS < I < Br < NO_3 < Cl < OCO.CH_3 < HPO_4 < SO_4$$
$$NH_4 < K < Na < Li.$$

The protein used in these experiments was white of egg. According to PAULI certain ions have a precipitating action and others a solvent action. The action of a salt corresponds to the algebraic sum of the action of the ions.[1] PAULI has attempted to associate the precipitation ability of the salts in relation to their action upon the coagulation temperature, but without any positive results.[2]

Nevertheless SPIRO [3] has shown that the kind of protein as well as its concentration are of importance for the precipitation action, and HÖBER [4] has recently shown that the series $I < Br < Cl < SO_4$ and $Li < Na < K < Rb < Cs$ is valid in alkaline reaction, but that the series is reversed in acid reaction. In nearly neutral reaction irregularities in the ion series occur which can be considered as a transition series between the two just-mentioned series. That the reaction must be of great importance in the precipitation of proteins seems very probable in consideration of the fact that the proteins take a decided electric charge on the addition of acid or alkali. In regard to the precipitation by salts of the heavy metals, the hydrophile colloids do not seem to differ essentially from the suspension colloids.[5]

On boiling a protein solution the protein suffers an irreversible change and under certain circumstances flocks out. Boiled but not flocked egg-white behaves with precipitating substances, like a suspension colloid.[6] In regard to the precipitation of proteins see Chapter II.

Theories of Precipitation Phenomena.

At least for the suspension colloids there is no question that they are flocked out by ions which carry an electric charge opposite to the colloid particles, and also by other colloids having an opposite charge. This fact follows from HARDY'S theory, according to which the flocking out is a neutralization process in which the charge of the colloid is just neutralized and the colloid therefore precipitates.[7] The mixture formed on precipitation has been shown to be electrically neutral (isoelectric) as the precipitated particles show no cataphoresis. In this manner it is easily understood that polyvalent ions have a stronger precipitating action than monovalent, as the electrical charge in, for

[1] Hofmeister's Beiträge, **3**, 225 (1902).
[2] Pflüger's Arch., **78**, 315 (1899).
[3] Spiro, Hofmeister's Beiträge, **4**, 300 (1903).
[4] *Ibid.*, **11**, 35 (1908).
[5] Pauli, *Ibid.*, **6**, 233 (1905).
[6] Hardy, Proc. Roy. Soc., **66**, 110 (1900).
[7] Zeitschr. f. physik. Chem., **33**, 385 (1900).

example, a trivalent ion is three times greater than in a monovalent ion. Otherwise greater precipitation ability of polyvalent ions can also be explained by a greater hydrolytic cleavage of the salts (page 23).

The mechanism of the precipitation of the isoelectric solution accepted in HARDY'S theory is explained by BREDIG [1] as follows: At the boundary between suspended particles and solvent a certain surface tension exists which tries to diminish the total contact surface between the two media, which can happen by the small particles uniting to form larger ones, when flocking is brought about. The electrical charge of the particles acts against the surface tension so that equally charged particles repel each other. If the electrical charge is discharged, as takes place in the isoelectric point, then the surface tension reaches its highest value and the precipitation may occur.

The correctness of HARDY'S claim that precipitation occurs just in the isoelectric fluid is disputed on special grounds by BILLITZER. He believes that the ions have a much greater charge than the colloid particles. An ion collects the oppositely charged colloid particles around itself, and during these neutralization processes it may occur, that the entire complex may become so large as to become visual and on account of the gravity it precipitates out.

In general it can be stated that the stability of a colloid is greater the smaller, *cet. par.*, the particles are; as the probability that the number of particles sufficient for the precipitation is then less. With equal size of particles the stability of a colloid is dependent upon the size of the charge which the particles carry. Too weak and very strongly charged colloids are relatively more stable; the first because of the large number which must collect around an ion when flocking takes place and the second because the number of particles required for the neutralization is perhaps too small, so that the necessary size of the complex for precipitation is not attained.[2]

The findings of LINDER and PICTON [3] that when colloidal As_2S_3 is precipitated with $BaCl_2$ the solution becomes acid, and a small quantity of barium remains in the precipitate, corresponds to BILLITZER'S theory. This quantity of barium cannot be removed by water, but can be replaced by the corresponding cation by washing with a solution of another salt. According to BILLITZER in the mutual precipitation of colloids a quantity relation exists which is dependent upon the electrical charges [4] (see also page 22).

The fact that the precipitation of colloids is a manifestation of processes which occur in a homogeneous medium, makes the understanding of these especially difficult. If, as is generally accepted, we consider

[1] Anorganische Fermente (1901), 15.
[2] Zeitschr. f. physik. Chem. Soc., **45**, 327 (1904); **51**, 129 (1905).
[3] Journ. Chem. Soc., **67**, 63 (1895).
[4] Zeitschr. f. physik. Chem., **51**, 141 (1905).

the colloid solution as a homogeneous fluid of suspended solid or fluid particles, then in the "solution" there occur at least two special constituents, separated from each other—the colloid particles and the solvent. This is expressed as follows: the system contains two *phases*. The solvent is often more correctly called the *dispersion means* and the colloid particles called the *disperse phase*. If to such a system a new substance is added, then the reaction which follows, depends essentially upon the division of the new substance between the two phases. In regard to the possible division two cases will be presented:

1. The process can be similar to the division of a soluble substance between two solvents. If a substance is brought in contact with two solvents at the same time, then it divides itself so that the relation between the concentration in the two solvents remains the same but independent of the total quantity of the dissolved substance. If the quantity of substance in each 100 cc. of the two solutions 1 and 2 is designated by c_1 and c_2, then it follows that $\frac{c_1}{c_2}=k$ where k is a constant.[1] The first example where this law was shown to be correct was the division of succinic acid between water and ether (BERTHELOT and JUNGFLEISCH[2]). This law was also shown to be true for the division of a gas between a gaseous and a fluid phase, i.e., for the absorption of a gas in a fluid (HENRY'S law of absorption). The conditions for the correctness of this law are that the temperature remains the same in experiments with different quantities of substance as well as that the substance has the same molecular size in the two phases.

2. In those cases where finely divided solids take up dissolved substances or gases the division is generally not independent of the total quantity of the dissolved substance or of the gas. This is often called *adsorption*.[3] For example, if we are dealing with the adsorption of a dissolved substance by a finely divided solid occurring in a solution, then a greater percentage is taken up from a dilute solution than from a concentrated one. On increasing concentration the adsorbed fraction becomes continuously less so that the absolute quantity taken up reaches a maximum which corresponds to the greatest adsorption ability of the solid body.

This is expressed by the formula $\frac{c_1 n}{c_2}=k$, where c_1 and c_2 indicate the concentration of the solid body and in the solution; n and k are constants and indeed, n is

[1] Nernst, Zeitschr. f. physik. Chem., 8, 110 (1891).

[2] Ann. Chim. phys. (4), 26, 396 (1872).

[3] It must be remarked that in the older literature oftentimes no difference was made between adsorption, and absorption, in which case both processes were included under the name absorption.

always >1. (If $n=1$ then the formula would be $\frac{c_1}{c_2}=k$ and we would be dealing with a so-called solid solution.)

APPLEYARD and WALKER[1] have studied the adsorption of organic acids from aqueous and alcoholic solutions by means of silk; the division was found to correspond to the above formula for adsorption. FREUNDLICH[2] has also carefully tested the adsorption of crystalloids by carbon. From these experiments it was shown that the equilibrium could be quickly attained from both sides, i.e., that the process was readily reversible. The above-given formula was found sufficiently accurate for the case where only the total quantity of the dissolved (to adsorb) substance varied. The series in which the organic acids were adsorbed by silk, as found by APPLEYARD and WALKER, were pratically the same as with carbon. The influence of temperature was slight.

According to KÜSTER,[3] the combination between starch and iodine is to be considered as an adsorption compound, and BILTZ[4] finds for the division of As_2O_3 between iron hydroxide (1) and water (2) the formula $\frac{c_1}{c_2}=0.631$.

The theoretical foundations for the adsorption phenomenon are not especially clear. Generally the adsorption is considered as connected with segregation and surface tension phenomenon. At the contact surface between a solid body and solution a surface tension exists which is considered as positive, i.e., this attempts to diminish the contact surface. The surface energy used thereby tends to be a minimum potential energy. As the product from size of surface and surface tension are the same, and as the first cannot change, the surface energy can only be diminished by a reduction of the tension. If, therefore, the tension is diminished by increasing the concentration of a substance dissolved in a fluid, then this substance tries to collect itself at the surface in greater concentration than in other parts of the fluid (OSTWALD,[5] FREUNDLICH[6]). In regard to the surface tension of solid-fluid we only know that it is positive, but can otherwise show great differences (OSTWALD,[7] HULETT[8]). According to this theory the facts are that certain solid substances possess the ability of adsorbing dis-

[1] Journ. Chem. Soc., **69**, 1334 (1896).
[2] Ueber die Adsorption in Lösungen, Leipzig (1906).
[3] Ann. d. Chem. u. Pharm., **283**, 360 (1894).
[4] Ber. d. d. chem. Gesellsch., **37**, 3138 (1904).
[5] Lehrb. d. allg. Chem., 2. Aufl., 2. Bd., 3. Teil, 237 (1906).
[6] Ueber Adsorption in Lösungen, 50–51.
[7] Zeitschr. f. physik. Chem., **34**, 495, 1900.
[8] *Ibid.*, **37**, 385 (1901).

solved bodies, and for this reason the adsorbed substance lowers the surface tension of the solid-fluid, and indeed, the more the greater concentration in which it occurs. That especially carbon and colloid substances are adsorption bodies lies in the fact that they have an especially large surface due to their finely divided state or porosity, which therefore, *cet. par.*, must give them a great surface energy.

That proteins, on precipitation, carry down other bodies with avidity is well known; inorganic hydrogels also take up dissolved substances with energy. The curves obtained for the latter process by VAN BEMMELEN [1] show a close analogy with the characteristic curves for the adsorption compounds. It often occurs that the body taken up homogeneously saturates the hydrogel, in which case $\frac{c_1}{c_2}=k$, and a sort of solid solution is the result. In certain cases, undoubtedly, chemical combinations with quite positive conditions are formed.

The precipitation of colloids by electrolytes has also been discussed by FREUNDLICH [2] from the standpoint of the adsorption hypothesis. Thus, for the precipitation ability of an electrolyte, the electric charge of the precipitating ion comes first into consideration and secondly, the ability of the precipitating colloid to adsorb the same. According to MOORE and ROAF[3] the salts of the red corpuscles are retained as adsorption compounds (adsorpates) by the proteins.

Thus far only the adsorption of crystalloids has been considered. Colloids are also taken up by solid substances or by other colloids. Still in these cases the conditions are more complicated than in the above-mentioned adsorption phenomena, as the combinations formed are in special cases irreversible or gradually become irreversible. It is well known that carbon takes up colloidal colored substances, and we have numerous examples of the combination of dissolved colloids with solid colloids in technology. BILTZ [4] has been able to show that many dyeing processes are to be considered as adsorption phenomena, and later FREUNDLICH and LOSEV [5] have measured the adsorption of basic and acid pigments by carbon and also by fibers (wool, silk, cotton), and have shown the correspondence of the two processes. With the basic pigments, which were used as salts, a splitting occurred into a pigment base, which was taken up by the fibers as well as by carbon, and an acid which quantitatively remained behind. This is similar to the cleavage which precipitating electrolytes undergo in the precipitation of the suspension colloids (see page 26).

[1] Zeitschr. anorg. Chem., **23**, 111, 321 (1900).
[2] Zeitschr. f. Chem. u. Ind. d. Koll., **1**, 321 (1907).
[3] Bioch. Journ., **3**, 55 (1908).
[4] Ber. d. d. chem. Gesellsch., **37**, 1766 (1904); **38**, 2963, 2973, 4143 (1905).
[5] Zeitschr. f. physik. Chem., **59**, 284 (1907).

Tanning is also brought about by adsorption processes, as the prepared skins adsorb the tanning substance.[1]

The precipitation of portein by adding finely divided solids (carbon, kaolin[2]) or by suspended solids (mastic[3]) precipitated in the liquid, as well as the action of protective colloids as already mentioned are also due to adsorption processes. The precipitation of protein, which occurs on shaking the protein solution with liquids, in which the protein is not soluble, is also to be considered as a surface tension action (RAMSDEN[4]).

BECHHOLD,[5] in his above-mentioned experiments on the filtration of colloids, has observed conditions which he considers as adsorption phenomena. Under certain circumstances a colloid can prevent the filtration of another colloid. A filter which was permeable for colloidal As_2S_3, but retained colloidal Prussian blue, did not allow a clear mixture of the two to pass through. The particles of As_2S_3, were adsorbed by the particles of Prussian blue, and could therefore not pass through the filter.

Gels. We have often mentioned gels or jellies (page 14). Only certain colloids can occur in the form of gels. Certain gels are spontaneously formed in sufficiently concentrated solutions (silicic acid, certain metallic hydroxides) and these do not redissolve in water. Other gels, like gelatin and agar, are formed on cooling of the hot, concentrated solutions, and are again soluble in water.

According to HARDY[6] the gel formation of gelatin is to be considered as a segregation process whereby a separation into two fluids occurs, one of which solidifies. The two phases are only differentiated by the microscope, and the chemical testing of the theory fails because of the circumstances that the two phases cannot be analyzed separately. In opposition to this PAULI claims that the gel passes through all of the intermediary steps into the corresponding sol and is therefore homogenous in the same sense as these.[7]

When gels are freed from water by evaporation or in other ways, they show a special ability to take up water, which is brought about by different processes which are included in the ordinary term *imbibition*. The views on this imbibition are indefinite. Surface phenomena play a rôle here. According to VAN BEMMELEN[8] the water is not chemi-

[1] See Zeitschr. f. Chem. u. Ind. d. Koll., **2**, 257 (1908).
[2] Bioch. Zeitschr., **5**, 365, 1907.
[3] *Ibid.*, **2**, 219 (1906); **3**, 109 (1906).
[4] Zeitschr. f. physik. Chem., **47**, 343 (1904).
[5] *Ibid.*, 60, 299 (1907).
[6] *Ibid.*, **33**, 326 (1900).
[7] Bioch. Zeitschr., **18**, 367 (1909).
[8] Zeitschr. anorg. Chem., **13**, 233 (1896); **20**, 185 (1899).

cally combined in definite proportions, but the quantity continually changes with the temperature and the vapor pressure. On the other hand, the imbibition stands in close relation to the osmotic pressure which is evident, if we define the osmotic pressure of a substance as its ability to attract water. The relation between imbibition and osmotic pressure is still closer in those cases when the substance finally is dissolved in water.

If a hydrogel is placed in a salt solution instead of in pure water, the imbibition phenomena essentially change. This was first studied by HOFMEISTER,[1] using gelatin plates. The process is rather complicated, as salt is taken up by one side of the gelatin plate and water by the other, and the taking up of water is influenced by the quantity of salt taken up. It has also been found that when gelatin plates are treated with solutions of increasing concentration of the same salt, the taking up of salt increases at first with the salt concentration, then becomes slower, and attempts to reach a maximum and then remains almost stationary. As long as the taking up of salt increases, the quantity of water passing into the gelatin also increases; when the salt fails to pass then the water also ceases to pass. It has also been found that the maximum of salt absorption for sulphate, tartrate and citrate can be attained with much lower molecular concentrations than with chloride, nitrate and bromide. From this it follows that the sulphate, tartrate and citrate have a retarding action upon imbibition within certain limits of concentration, while the chloride, nitrate and bromide have an accelerating action.

PAULI[2] has investigated the influence of salt solutions upon the solidification and melting-point of gelatin. If the salts are arranged in the order of their ability to lower the solidification point of gelatin we come to the series sulphate, citrate, tartrate, acetate (water), chloride, chlorate, nitrate, bromide, iodide. This series corresponds well with that of HOFMEISTER.

Acids and alkalies exert a special influence upon gelatin, as they both, in very dilute solutions, strongly accelerate imbibition (SPIRO,[3] WO. OSTWALD[4]). From the previously mentioned investigations of LILLIE, on the osmotic tension of gelatin solutions, it was found that the addition of acids and alkalies increased it (page 17).

Since GRAHAM'S fundamental experiments it was believed that colloidal sols could not diffuse into gels while crystalloids could pass just

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[1] Arch. f. exp. Pathol. u. Pharm., 28, 210 (1891).
[2] Pflüger's Arch., 71, 333 (1898).
[3] Hofmeister's Beiträge, 5, 276 (1904).
[4] Pflüger's Arch., 108, 563 (1905).

as quickly into gels as into pure water. Nevertheless, SPIRO [1] has observed that dissolved ovalbumin as well as hæmoglobin could pass into gelatin plates. On the other hand K. MEYER [2] as well as BECHHOLD and ZEIGLER [3] have found that the distance passed by a crystalloid in gelatin may be much shorter than in pure water. In such experiments no doubt adsorption processes must be considered.

III. CATALYSIS.

When two bodies which can act chemically upon each other are brought together the reaction generally takes place so fast that it cannot be measured. In other cases, by special means, we can observe how the reaction gradually proceeds. When cane-sugar is inverted by weak acid, the decrease in the rotation of the solution can be followed with the polariscope; and when an ester is decomposed by alkali the quantity of still free alkali can be determined by titration. The quantity of substance measured in gram-molecule per liter (molè) which is decomposed in the unit of time, is called the *reaction velocity* of the system. The so-called law of *mass action*, as proposed by GULDBERG and WAAGE, states that the reaction velocity is every moment proportional to the molecular concentration of the reacting bodies. A mixture of alcohol and acetic acid is transformed into acetic ether and water, especially in the presence of some mineral acid. If the molecular concentration of the alcohol and acid be designated by C_A and C_S, then according to the law of mass action the reaction velocity is $v_1 = k_1 . C_A \cdot C_S$, where k_1 indicates a constant which is independent of the quantity of reacting substances and the time limit is so short that the concentration can be considered as constant. This reaction, like many others, is reversible, i.e., two reactions occur simultaneously: one between the alcohol and acetic acid, producing acetic ether and water, and second, between acetic ether and water, re-forming alcohol and acetic acid. This is expressed as follows:

$$C_2H_5.OH + HO.CO.CH_3 \rightleftarrows C_2H_5.O.CO.CH_3 + H_2O.$$

The velocity of reaction when it passes from left to right is called v_1. If the velocity in the reverse reaction is called v_2 and the molecular concentration of the acetic ether and water is called C_E and C_W, then we obtain $v_2 = k_2 \cdot C_E \cdot C_W$. At the beginning when C_E as well as

[1] Hofmeister's Beiträge, **5**, 294 (1904).
[2] *Ibid.*, **7**, 393 (1905).
[3] Zeitschr. f. physik. Chem., **56**, 105 (1906).

$C_W=0$, the velocity of the ester formation is expressed by the formula $v_1=k_1\cdot C_A\cdot C_S$; afterward it is expressed by the difference v_1-v_2 or $k_1\cdot C_A\cdot C_S-k_2\cdot C_E\cdot C_W$. Of the two reaction velocities v_1 and v_2 at the begining v_1 always diminishes while v_2 increases. When $k_1\cdot C_A\cdot C_S=k_2\cdot C_E\cdot C_W$ is attained, then the velocity of both reactions is the same; no measurable decomposition occurs and the system is in equilibrium. The equilibrium condition is the same irrespective of whether we start from alcohol+acetic acid or from the corresponding quantity of acetic ether+water. On equilibrium it is

$$k_1.C_A.C_S=k_2.C_E.C_W \text{ or } \frac{C_A.C_S}{C_E.C_W}=\frac{k_2}{k_1}=K.$$

K is called the equilibrium constant; as is apparent it can be determined in two ways—either from the concentration of the reacting bodies when equilibrium is present or from the velocity coefficient k_1 and k_2 as determined in a manner given below.

In the above-mentioned transformation of alcohol and acetic acid these two bodies are simultaneously used up. The reaction is therefore called bimolecular, and a reaction is called mono-, bi-, tri-, etc., molecular according to the number of the kinds of molecules which diminish their concentration thereby.[1]

BERZELIUS[2] found that certain bodies by their mere presence, and not by their affinity, have the power of awakening the dormant affinity at a certain temperature, i.e., the power of starting a reaction. These phenomena were called *catalytic* by BERZELIUS.

According to OSTWALD[3] catalysis is the acceleration (or retardation) of a slow-proceeding chemical change by the presence of a foreign body. That body which influences a reaction in this manner is called a *catalyst*. It does not itself undergo any appreciable change by the reaction.

Catalytic reactions have been studied, especially by WILHELMY,[4] VAN'T HOFF,[5] OSTWALD,[6] ARRHENIUS[7] and BREDIG.[8] Of all other substances the acids and alkalies seem to act most catalytic. A well-known

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[1] It is assumed here that of every kind of molecule one molecule of each takes part in the reaction.

[2] Berzelius, Årsberättelse om framstegen i Fysik och Kemi., 18, p. 245 (1836).

[3] Lehrb. d. allg. chem. 2. Aufl. II., 1, 515.

[4] Poggendorff's Ann., 81, 413 (1850).

[5] Études de dynam. chim. (1884).

[6] Lehrb. d. allg. Chem., 2. Auf. II, 2, 199.

[7] Zeitschr. f. physik. Chem., 4, 226 (1889).

[8] Anorganische Fermente (1901); Bioch. Zeitschr., 6, 283 (1907).

example is the inversion of cane-sugar by means of acid. This reaction is monomolecular because only the cane sugar is consumed. If the concentration of the cane-sugar at the beginning is C moles, and if x moles are transformed in t time, then at that time there are $(C-x)$ moles remaining. If dx indicates the quantity which is transformed in dt time, then the reaction velocity is $\frac{dx}{dt}$. According to the law of mass action this is at every moment proportional to the concentration of the decomposing substance, or

$$\frac{dx}{dt}=k.(C-x). \quad \ldots\ldots\ldots \quad (1)$$

For practical use this equation is integrated into the following:

$$k=\frac{1}{t}\text{nat. log.}\frac{C}{C-x}. \quad \ldots\ldots\ldots \quad (2)$$

If the theoretical considerations upon which this formula is based are correct, then the x values determined by the polariscope after various times must give the same figure for k. This is indeed the case.[1] k is called the *velocity coefficient* (also velocity constant or specific reaction velocity). If in the equation (1) $C-x$ or the concentration of the still undecomposed cane-sugar $=1$, then the equation becomes $\frac{dx}{dt}=k$, from which it follows that k indicates the reaction velocity if the concentration of the substrate could be kept the entire time at $=1$.

In these experiments k retains the same value. If in different experiments the quantity of catalyst (acid) varies, then the obtained value for k is proportional to the concentration of the H ions. This is so prominent that the catalytic action of acids is due to the H ions (Arrhenius[2]). Still irregularities occur as the anions of acids as well as of salts present can under certain circumstances influence the action of H ions (see page 70).

Fränkel[3] has recently studied the decomposition of diazoacetic ether under the influence of different acids. The reaction is as follows:

$$N_2 : HC.CO.O.C_2H_5+H_2O=HO.CH_2.CO.O.C_2H_5+N_2.$$

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[1] See Poggend. Ann., 81, 413 and 499 (1850).
[2] Zeitschr. f. physik. Chem., 4, 226 (1889).
[3] *Ibid.*, 60, 202 (1907).

The progress of the reaction can be determined by measuring the nitrogen set free. The following figures explain the results:

Acid.	Conc. of the Acid in Mol. per Liter.	C_H. Conc. of the H ions by Electric Conductivity.	K Velocity Coefficient.	$\frac{K}{C_H}$
Nitric acid	0.001820	0.001820	0.0703	38.7
	0.000909	0.000909	0.0346	38.0
Picric acid	0.000909	0.000909	0.0356	39.2
	0.000364	0.000364	0.0140	38.3
m-Nitrobenzoic acid	0.009900	0.001680	0.0632	37.7
Fumaric acid	0.003640	0.001460	0.0571	39.1
Succinic acid	0.009090	0.000724	0.0285	38.5
Acetic acid	0.018200	0.000563	0.0218	38.7

As $\frac{K}{C_H}$ for the different acids and different quantities of acid is the same, then the velocity coefficient is here also proportional to the concentration of the H ions.

As the catalytic action of acids is caused by the H ions, so are the catalytic properties of bases due to the OH ions. The first determined case of this kind was the transformation of hyoscyamine into the stable atropine.[1]

KOELICHEN [2] has studied a specially pretty case of the catalytic action of OH ions in the decomposition of diacetonalcohol into acetone:

$$CH_3.CO.CH_2.C(CH_3)_2.OH = 2CH_3.CO.CH_3.$$

The reaction is reversible, and from the following table it is seen that the velocity constant for various concentrations of the same catalyst remains the same as well as by using different bases.

Catalyst.	Conc. of the Catalyst.	Velocity Constant.
Piperidine	0.1090	0.038
Triethylamine	0.4900	0.036
Ammonia	0.5500	0.038
Tetraethylammonium hydroxide	0.0760	0.037
	0.0076	0.037
Sodium hydroxide	0.0725	0.036
	0.0072	0.035

By this a rule which VAN'T HOFF and OSTWALD [3] proved by thermodynamic means, is substantiated, namely, that the equilibrium at constant temperature does not change with the quantity and kind of catalyst when the catalyst is not changed by the reaction.

Among other kinds of ions which act as catalysts we must mention (1) iodine ions, which decompose H_2O_2 in proportion to their concentration,[4] and (2) cyan-

[1] Ber. d. d. chem. Gesellsch., **21**, 2777 (1888).
[2] Zeitschr. f. physik. Chem., **33**, 129 (1900).
[3] Van't Hoff, Vorlesungen, **1**, 211.
[4] Walton, Zeitschr. f. physik. Chem., **47**, 185, 1904.

ions, which transform benzaldehyde into benzoin according to the following equation:

$$2C_6H_5COH = C_6H_5CO.CH(OH).C_6H_5.$$ [1]

If those bodies which accelerate a reaction are to be considered as catalysts, then certainly the solvents must belong to the catalytes. Attention must be called to the enormous influence which the solvent can exert upon the velocity of a reaction under otherwise equal conditions. Thus MENSCHUTKIN [2] found for the reaction

$$(C_2H_5)_3.N + C_2H_5.I = (C_2H_5)_4.N.I.,$$

the following velocity in different solvents:

Hexane	0.00018
Heptane	0.000235
Xylene	0.00287
Benzene	0.00584
Ethyl alcohol	0.03660
Benzyl alcohol	0.13300

Recently BREDIG and FAJANS [3] have been able to show that an optically active solvent can help in the decomposition of optical antipodes to a varying extent. Of the optical antipodes of campho-carboxylic acid, the *d*-form is 17 per cent more quickly decomposed than the *l*-form, when they are dissolved in nicotine or when nicotine is present, dissolved with the catalyte, while in an optically indifferent solvent and without any nicotine the catalyte decomposes both forms with equal rapidity. The reaction proceeds differently with or without catalyst, and hence the catalyst brings about changes in reaction other than those of velocity. It is apparent that this does not conform with OSTWALD'S definition of a catalyst (page 33). It must be mentioned that BREDIG and FISKE have been able to perform the asymmetric synthesis of benzaldehyde and hydrocyanic acid by means of quinine and quinidine as catalysts (page 60).

Catalysis in Heterogeneous Systems. The above-treated catalytic processes all occur in homogenous systems, i.e., the systems which by mechanical means cannot be separated into different constituents. In heterogeneous systems with phases which can be separated from each other by mechanical means, catalytic reactions can also occur, and indeed, in such cases the substances taking part in the reaction and the catalyst occur in different phases. Such a reaction is the union of detonating gas, the synthesis of SO_3 (from SO_2 and O), and the decomposition of H_2O_2 by platinum. In case the system is two-phased, and the reaction takes place only at the boundary between both phases, or in the one we can differentiate two simple limits:

1. The accumulation of the bodies which are necessary for the reaction at the proper place takes such a short time that in comparison

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[1] Stern, *ibid.*, **50**, 513 (1905).

[2] *Ibid.*, **6**, 41 (1890).

[3] Ber. d. d. chem. Gesellsch., **41**, 752 (1908).

with the real chemical reaction it can be neglected. In these cases the reaction velocity behaves similarly to a homogeneous system.[1]

2. The chemical reaction occurs at a rate which in comparison with the time necessary for the accumulation can be neglected. In this case the time necessary can be generally compared with a diffusion process.[2]

The catalytic processes in heterogeneous systems have excited interest since BREDIG[3] showed that the colloidal metals prepared by him showed catalytic properties. The best-studied process is the decomposition of H_2O_2 by colloidal platinum, gold, and other metals or oxides (MnO_2, PbO_2). Attention must be called to the small quantity of catalyst sufficient to decompose H_2O_2. The action of 1 gram atom platinum in 70 million liters of reaction mixture has been detected. The decomposition of H_2O_2 by platinum catalyst in nearly neutral or faintly acid solution has been shown to be a monomolecular reaction.

Still certain differences occur from the conditions formed in the homogeneous catalysis. At one time in certain experiments the value for k rises considerably during the catalysis, and secondly, k is not proportional to the ferment concentration, but rises more quickly than this.

In connection with these experiments BREDIG has expressed the view that an analogy exists between the catalytic processes of the inorganic world and the enzyme action of the organic.

The following important facts give support to BREDIG'S view:

1. In both cases we are dealing with catalytic processes; the metallic sol and the enzyme are active in very small quantities and during the reaction they do not undergo any appreciable change.

2. In the decomposition of H_2O_2 by platinum sols or by the enzyme hæmase, the reaction is monomolecular.

3. The action of metallic sols as well as enzymes is paralyzed by certain poisons (HCN, H_2S).

4. Both classes of bodies are colloid substances and possess an enormous surface upon which their catalytic action depends.

According to NEILSON,[4] ethyl butyrate, salicin and amygdalin are decomposed by platinum black as well as by enzymes.

IV. ENZYMES.

Chemical Processes in Plants and Animals. It follows from the law of the conservation of matter and of energy that living beings, plants and animals, can produce neither new matter nor new energy. They

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[1] Goldschmidt, Zeitschr. f. physik. Chem., **31**, 235 (1899).

[2] Nernst and Brunner, *ibid.*, **47**, 52 and 56 (1904).

[3] Anorganische Fermente, Leipzig, 42 (1901).

[4] Amer. Journ. of Physiol., **10**, 191 (1904); **15**, 148 (1906).

are only called upon to appropriate and assimilate material already existing and to transform it into new forms of energy.

Out of a few relatively simple combinations, especially carbon dioxide and water, together with ammonium compounds or nitrates, and a few mineral substances, which serve as its food, the plant builds up the extremely complicated constituents of its organism—proteins, carbohydrates, fats, resins, organic acids, etc. The chemical work which is performed in the plant must, therefore, in the majority of cases, consist in syntheses; but besides these, processes of reduction take place to a great extent. The radiant energy of the sunlight induces the green parts of the plant to split off oxygen from the carbon dioxide and water and this reduction is generally considered as the starting-point in the syntheses that follow. According to a hypothesis suggested by A. BAEYER,[1] formaldehyde is first produced, $CO_2+H_2O=CH_2O+O_2$, which by condensation is transformed into sugar. From the sugar other bodies can then be built up.

With the aid of the silent electric discharge W. LOEB[2] has succeeded in obtaining from carbon dioxide and water, formaldehyde, and as a product of polymerization, also glycolaldehyde, $CH_2OH.CHO$, from which sugar can be readily produced. Still the conditions under which these bodies were formed cannot be applied to the conditions in the plants. The investigations of USHER and PRISTLEY[3] are of greater interest in that they show the formation of formaldehyde in the photolytic decomposition of moist carbonic acid in the presence of chlorophyll. These investigations also do not seem to be entirely free from exception. The conception as to the formation of sugar from formaldehyde is also often different from that explained by v. BAEYER'S idea, and his view as to the assimilation of carbonic acid constitutes a hypothesis which requires further proof. The essentials of this hypothesis, namely, a formation of formaldehyde with a subsequent sugar formation by condensation of the aldehyde groups, is still very generally accepted as probably correct. Independent of the ways and means of how the assimilation processes in the plants originate, it is obvious that the free, radiant energy of the sun is hereby bound and stored in a new form, as chemical energy, in the combinations formed by the syntheses.

In animal life the conditions are not the same. Animals are dependent either directly, as the herbivora, or indirectly, as the carnivora, upon plant-life, from which they derive the three chief groups of organic nutritive matter—proteins, carbohydrates, and fats. These bodies, of which the protein substances and fats form the chief mass of the

[1] Ber. d. d. chem. Gesellsch., **3**.
[2] Zeitschr. f. Electrochem., **12**.
[3] Proc. Roy. Soc. London, **78**, Series B.

animal body, undergo within the animal organism a cleavage and oxidation, and yield as final products exactly the above-mentioned chief components in the nutrition of plants, namely, carbon dioxide, water, and ammonia derivatives, which are rich in oxygen and have little energy. The chemical energy, which is partly represented by the free oxygen and partly stored up in the above-mentioned more complex chemical compounds, is transformed into other forms of energy, principally heat and mechanical work. While in the plant we find chiefly reduction processes and syntheses, which by the introduction of energy from without produce complex compounds having a greater content of energy, we find in the animal body the reverse of this, namely, cleavage and oxidation processes, which, as we used to state, convert chemical tension into living force.

This difference between animals and plants must not be overrated, nor must we consider that there exists a sharp boundary line between the two. This is not the case. There are not only lower plants, free from chlorophyll, which in regard to chemical processes represent intermediate steps between higher plants and animals, but the difference existing between the higher plants and animals is more of a quantitative than of a qualitative kind. Plants require oxygen as peremptorily as do animals. Like the animal, the plant also, in the dark and by means of those parts which are free from chlorophyll, takes up oxygen and eliminates carbon dioxide, while in the light the oxidation processes going on in the green parts are overshadowed or hidden beneath the more intense reduction processes. As in the animal, we also find a heat production in fermentation produced by plant organisms; and even in a few of the higher plants—as the *aroideæ* when bearing fruit—a considerable development of heat has been observed. On the other hand, in the animal organism, besides oxidation and splitting, reduction processes and syntheses also take place. The contrast which seemingly exists between animals and plants consists merely in that in the animal organism the processes of oxidation and splitting are predominant, while in the plant chiefly those of reduction and synthesis have thus far been studied.

WÖHLER[1] in 1824 was the first to observe an example of the SYNTHETICAL PROCESSES within the animal organism. He showed that when benzoic acid is introduced into the stomach, it reappears as hippuric acid in the urine after combining with glycocoll (aminoacetic acid). Since the discovery of this synthesis, which may be expressed by the following equation:

$$\underset{\text{Benzoic acid}}{C_6H_5.COOH} + \underset{\text{Glycocoll}}{NH_2.CH_2.COOH} = \underset{\text{Hippuric acid}}{NH(C_6H_5.CO).CH_2.COOH} + H_2O,$$

[1] Berzelius, Lehrb. d. Chemie, übersetzt von Wöhler, 4, p. 356, Abt. 1, Dresden (1831).

and which is ordinarily considered as a type of an entire series of syntheses occurring in the body, where water is eliminated, the number of known syntheses in the animal kingdom has increased considerably. Many of these syntheses have also been artificially produced outside of the organism, and numerous examples of animal syntheses of which the course is absolutely clear will be found in the following pages. Besides these well-studied syntheses, there also occur in the animal body similar processes unquestionably of the greatest importance to animal life, but of which we know nothing with positiveness. We enumerate as examples of this kind of synthesis the re-formation of the red-blood pigment (the hæmoglobin), the formation of the different proteins from simpler substances, and the production of fat from carbohydrates. This last-mentioned process, the formation of fat from carbohydrates, is also an example of reduction processes which occur to a considerable extent in the animal body.

Certain reactions, which are either not reproduceable with dead material or are only possible under conditions which destroy the cells, belong to the chemical decompositions going on within the living organism. Thus the synthesis of glycogen or of protein has not been accomplished outside of the organism or without the aid of agents prepared by the cells. On the other hand proteins and starches can be split into simpler products without these agents, but for this purpose the action of acids or alkalies of a concentration which would kill the cells is necessary. In certain cases it is possible to bring about such reactions outside of the organism without any injurious effect upon the cells. This is accomplished by the aid of substances which are formed within the cells but have the power of being active after they have left the cells. These substances have been called *enzymes* or *ferments*.

Enzymotic Processes. We must now mention a group of reactions which are more or less related to enzyme action.

In the first place the so-called *hydrolytic cleavage processes* in which complex substances are divided into simpler substances with the simultaneous decomposition of water and the taking up of its constituents. These processes are of the greatest importance in the digestion of the food-stuffs and for making them of value but they are also important for the metabolic processes in general. As examples of such cleavages we will mention the division of proteins into simpler products, the transformation of starch into sugar and the cleavage of neutral fats into the corresponding fatty acid and glycerin:

$$\underset{\text{Tristearin}}{C_3H_5(C_{18}H_{35}O_2)_3} + 3H_2O = \underset{\text{Glycerin}}{C_3H_5(OH)_3} + 3\underset{\text{Stearic Acid.}}{C_{18}H_{36}O_2}$$

The importance of the hydrolytic cleavage processes for digestion will be discussed in detail in Chapter VIII.

Other cleavage processes are certain so-called *fermentation processes*, which are connected with the presence of living organisms, fungi and bacteria of various kinds. Among these we include chiefly the alcoholic fermentation and butyric acid fermentation of carbohydrates. According to the view based upon PASTEUR'S investigations it has been generally considered that these processes are phases of the life of these organisms and the name *organized ferments* or *ferments* have been given to such organisms, especially to the ordinary yeast fungus.

A ferment, according to this view, is a living organism. By the name enzyme, as introduced by KÜHNE, we mean a product of the chemical processes in the cells, which is active without the life of the cell and which can be separated from the cell. The decomposition of invert sugar into carbon dioxide and alcohol in fermentation is considered as a fermentative process closely connected with the life of the yeast fungus. The inversion of cane-sugar previous to fermentation is on the contrary, an enzymotic process which is brought about by a body or mixture of bodies which are formed in the fungus and which can be removed from the fungus and are still active after the death of the fungus. Consequently ferments and enzymes are capable of manifesting a different behavior toward certain chemical reagents. Thus there exist a number of substances, among which we may mention arsenious acid, phenol, toluene, salicylic acid, boracic acid, sodium fluoride, chloroform, ether, and protoplasmic poisons, which in certain concentration kill ferments, or at least retard their action, but which do not noticeably impair the action of the enzymes.

The above view as to the difference between ferments and enzymes has lately been essentially shaken by the researches of E. BUCHNER[1] and his pupils. He has been able to obtain from beer-yeast, by grinding and strong pressure, a cell-fluid rich in protein, and which when introduced into a solution of a fermentable sugar caused a violent fermentation. The objections raised from several sides that the fluid expressed still contained dissolved living cell substance has been so successfully answered by BUCHNER and his collaborators that there is at present no question that alcoholic fermentation is caused by a special enzyme or mixture of enzymes called *zymase*, which is formed in the yeast-cell.

As from the yeast-cells so also from other lower organisms, indeed

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[1] E. Buchner, Ber. d. deutsch. chem. Gesellsch., **30** and **31**; E. Buchner and Rapp, *ibid.*, **31**, **32**, **34**; H. Buchner, Stizungsber. d. Gesellsch. f. Morphol. u. Physiol. in München, **13** (1897), part 1, which also contains the discussion on this topic. See also E. and H. Buchner and M. Hahn, Die Zymasegärung, München (1903); Stavenhagen, Ber. d. deutsch. chem. Gesellsch., **30**; Albert and Buchner, *ibid.*, **33**; Buchner, *ibid.*, **33**; Albert, *ibid.*, **33**; Albert, Buchner, and Rapp, *ibid.*, **35**; in regard to the opposed views see Macfadyen, Morris, and Rowland, *ibid.*, **33**; Wroblewski, Centralbl. f. Physiol., **13**, and Journ. f. prakt. Chem. (N. F.), **64**.

from the lactic-acid bacilli and beer vinegar bacteria, it is possible to separate the specific fermentative principle of these organisms from the living organism and to bring about changes with the dead organism (E. BUCHNER, and MEISENHEIMER and GAUNT, HERZOG [1]). The question whether there exist ferment processes which, in PASTEUR'S sense, are the result of the biological phenomena connected with the metabolism of the micro-organism and which we can directly identify with the life processes, is very difficult to answer; hence for the present we have no foundation for a sharp differentiation between the organized ferments and enzymes. The metabolic processes of the living organisms which we recognize as fermentation phenomena must as a rule be ascribed to enzymes acting within the cell. If such processes are closely connected with the life of the cell, then this is explained in part by the fact that this special enzyme is produced only by living cells and in part by the fact that it cannot be separated from the living cells or that it is readily destroyed on the death of the cell. The names enzyme and ferment are now generally used in the same sense.

Formerly the view was generally accepted that ANIMAL OXIDATION takes place in the fluids, while to-day we are of the opinion, derived from the investigations of PFLÜGER and his pupils,[2] that it is connected with the form-elements and the tissues. The question as to how this oxidation in the form-elements is induced and how it proceeds cannot be answered with certainty. On the other hand, it is accepted that the living protoplasm in some manner or other takes part, in which case the oxidation processes must cease with the life of the cells while on the other hand it has been found that certain oxidative processes can be brought about by means of catalyts in the ordinary sense as well as by enzymotic substances. If in the latter case the oxygen of the air is directly transported to the oxidizable substance then we call this a *direct oxidation*. Ordinarily the oxidative processes take place in the following way. First a peroxide is formed by taking up oxygen, like in the formation of hydrogen peroxide, H–O–O–H, which then transfers oxygen to the oxidizable substance by aid of the mentioned substances. In these cases the oxidation is *indirect*. All these oxidative processes will be treated in detail in Chapter XVI. At the same time also other enzymes will be discussed which decompose hydrogen peroxide, with the setting free of oxygen, without oxidizing at the same time. These

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[1] E. Buchner and J. Meisenheimer, Ber. d. d. chem. Gesellsch., **36**; 634 (1903); and Annal. d. Chem. u. Pharm., **349**; with Gaunt, *ibid.*, **349**; Herzog, Zeitschr. f. physiol. Chem., **37**.

[2] Pflüger, Pflüger's Archiv, **6** and **10**; Finkler, *ibid.*, **10** and **14**; Oertmann, *ibid.*, **14** and **15**; Hoppe-Seyler, *ibid.*, **7**.

have been called *catalases*. Also *reduction processes* will be mentioned which seem to be brought about by enzymes.

Besides these processes just mentioned the following processes, namely, *autolysis* and *putrefaction*, are to be considered as due to enzyme action entirely or in part.

If an animal organ is kept in water at 37° C. under conditions so that no micro-organisms are active then the organ gradually dissolves in great part under the influence of the contained enzymes. This process is called *autodigestion* or *autolysis*. The action of micro-organisms can be prevented either by removing the organ under strictly aseptic conditions or by allowing the digestion to take place in the presence of antiseptic substances (toluene, chloroform, etc.). As the animal organs consist chiefly of protein substances the autolysis consists chiefly in the action of enzymes which dissolve proteins. Autolysis was first observed and studied by SALKOWSKI and his pupils with liver, muscle and supra-renal capsule.[1] JACOBY then showed that the enzymes active in autolysis do not orginate in the digestive tract and are not identical with trypsin or pepsin.[2] BIONDI found that hydrochloric acid had a favorable influence upon the autolysis of the liver while HEDIN and ROWLAND[3] observed that the organic acids accelerate the autolysis of nearly all organs. This has been substantiated by several authors (WIENER, ARINKIN[4]). The findings of LANE-CLAYPTON and SCHRYVER[5] that the autolysis of the liver and kidneys begins only after a latent period of from two to four hours when the post mortem formation of acid is at its height, substantiates the influence of the acid reaction.

The autolysis is retarded to a great extent by an alkaline reaction. This is shown by the experiments of SCHWIENIG with the liver as well as those of HEDIN and ROWLAND with several other organs. HEDIN has also shown by experiments with various organs that a preliminary treatment with acetic acid markedly helps the autolysis in alkaline reaction, which for the spleen at least is explained by a destruction through the treatment of acetic acid of a substance which has an inhibiting action in alkaline solution. Such an inhibiting substance destroyed by acetic

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[1] Zeitschr. f. klin. Med., 1890, Suppl., Schwiening, Virchow's Arch., **136**, 444 (1894), Biondi, *ibid*, **144**, 373 (1896).

[2] A complete summary of the literature of intracellular enzymes and autolysis may be found in Jacoby, Ueber die Bedeutung der intrazellulären Fermente, etc., Ergebnisse der Physiologie, Jahrg. **1**, Abt. 1, 1902.

[3] Zeitschr. f. physiol. chem., **32**, 341, 531 (1901).

[4] Wiener, Centralbl. f. Physiol., **19**, 349 (1905); Arinkin, Zeitschr. f. physiol. Chem., **52**, 192 (1907).

[5] Journ. of Physiol., **31**, 169 (1904).

acid is also found in serum.[1] The serum also inhibits the autolysis of the liver (BAER, LONGCOPE and others)[2] and also the thymus under certain circumstances (RHODIN[3]).

Experience has shown that the post-mortem autolytic process may also be influenced by many other bodies and indeed in various ways. For example, according to HESS and SAXL, arsenious acid exerts a retarding action on the first stages of autolysis, while phosphorus accelerates it. IZAR as well as LAQUEUR and ETTINGER[4] obtained with small quantities of different arsenic preparations an accelleration of the autolysis and with larger amounts a retardation. LAQUEUR[5] obtained a retardation with oxygen and an acceleration with carbon dioxide. ASCOLI and IZAR[6] have thoroughly investigated the action of inorganic colloids upon autolysis. Radium rays as well as radium emanations accelerate post-mortem autolysis of normal as well as carcinoma tissues (WOHLGEMUTH, NEUBERG, LÖWENTHAL and EDELSTEIN[7]).

The products of the activity of the different enzymes dissolving proteins in autolysis have been studied by HEDIN and his collaborators, by studying the action of organ press juices upon protein added, or upon the protein contained in the juice. The same cleavage products were found as in the deep-seated cleavage of proteins in the digestive canal.[8] Similar investigations have also been carried on by LEVENE and JONES[9] who chiefly considered the decomposition of the nuclein substances. The combined action of various enzymes in autolysis also explains to us why, as especially shown by LEVENE and by JONES, the products obtained by the hydrolytic cleavage of an organ by means of an acid are somewhat different from those products produced on autolysis. In autolysis we are not only dealing with the cleavage of the proteins, but other enzymotic processes also occur such as the splitting of fats and carbohydrates, the splitting off of NH_2 groups from amino-acids, oxidations, reductions and perhaps also syntheses.

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[1] Hammarsten's Festschr., 1906.

[2] Baer, Arch. f. expt. Path. u. Pharm., **56**, 68 (1906); Longcope, Journ. med. Research, **13**, 45 (1908).

[3] Zeitschr. f. physiol. Chem., **75**, 197 (1911).

[4] Hess and Saxl, Zeitschr. f. expt. Path. u. Therapie, 5 (1908); Izar, Bioch. Zeitschr., **21**, 46 (1909); Laqueur and Ettinger, Zeitschr. f. physiol. Chem., **79**, 1 (1912).

[5] Zeitschr. f. physiol. Chem., **79**, 82 (1912).

[6] Bioch. Zeitschr., **17**, 361 (1909).

[7] Wohlgemuth, Berl. klin. Wochenschr., **26**, 704; Neuberg, Zeitschr. f. Krebsforschung, **2**, 171 (1904); Löwenthal and Edelstein, Bioch. Zeitschr., **14**, 484 (1908).

[8] Leathes, Journ. of Physiol., **28**, 360 (1902); Dakin, *ibid.*, **30**, 84 (1904); Hedin, *ibid.*, **30**, 155 (1904); Cathcart, *ibid.*, **32**, 299 (1905).

[9] Levene, Amer. Jour. of Physiol., **10**, **11**, **12** (1904); Jones, Zeitschr. f. physiol. Chem., **42**, 35 (1904).

It is at present impossible to state what part autolytic processes take in life under physiological conditions, and we can have only conjectures on this subject. In the autolysis of a removed organ or of one through which the blood is not flowing, the conditions in many ways are quite different from the conditions in life. The products which appear after weeks or months of autolysis, sometimes in very small quantities, do not give any clue to the nature of the vital processes, and conclusions must be drawn very carefully from these results.

For the present it is impossible to judge of the importance of the enzymes active in autolysis for physiological conditions, but this does not exclude the possibility that in normal cell life the enzymes play a very important rôle. Numerous observations show this to be true, and we tend more and more toward the view that the chemical transformations in the living cells are brought about by enzymes, and that these latter are to be considered as the chemical tools of the cells (HOFMEISTER and others.[1]).

From this standpoint the enzymes are of especial interest because to-day it is the general belief that nearly all chemical processes of great importance do not occur in the animal fluids, but on the contrary in the cells, which are the real chemical workshops of the organism. It is also chiefly the cells, which by their more or less active efficiency regulate the extent of the chemical processes and thereby also the intensity of the general metabolism. The following will be given as special examples of the action of such enzymes under pathological conditions. The changes of the liver and blood in acute phosphorus intoxication and in acute yellow atrophy of the liver, where we find, in the urine, the enzymotic decomposition products of the proteins.[2] Another example is the solution of pneumonic infiltrations by the enzymes of the migrated and inclosed leucocytes as studied by FR. MÜLLER,[3] and this is at the same time an example of *heterolysis*, i.e., of a solution or a destruction in an organ by enzymes not belonging therein but introduced from without. An autolysis, although not very marked, occurs in those organs or parts of organs which have not been normally nourished because of a disturbance in the circulation, and they are gradually consumed by this action. The part injured undergoes solution, while the healthy part remains unattacked. By this solvent action as well as by the formation of bactericidal bodies, as observed by CONRADI,[4] and of antitoxins

[1] F. Hofmeister, Die chemische Organisation der Zelle, Braunschweig, 1901.

[2] Jacoby, Zeitschr. f. physiol. Chem., **30**, 174 (1900).

[3] Verhandl. d. naturforsch. Gesellsch. zu Basel, 1901. See also O. Simon, Deutsch Arch. f. klin. Med., 1901.

[4] Hofmeister's Beiträge, 1.

(BLUM[1]) by means of autolysis, we can consider this autolysis as a remedy and perhaps also as a protective agent for the animal body. In this connection the investigations of BILLARD[2] must be mentioned where the autolytic fluid from the pig liver was strongly antitoxic toward viper poison, cobra poison, tetanus toxin and also toward cocaine, curare and strychnin.

As above stated, the chemical processes in animals and plants do not stand in opposition to each other; they offer differences indeed, but still they are of the same kind from a qualitative standpoint. PFLÜGER believes that there exists a blood-relation between all living cells of the animal and vegetable kingdoms, and that they originate from the same root. The animal body is a complexity of cells, hence study of the chemical processes must not only be made upon higher plants, but also upon unicellular organisms in order that we get a proper explanation of the chemical processes in the animal organism. Although a biochemical study of the micro-organisms is very important, we must bear in mind also the important rôle played by such organisms in animal life, chiefly as exciters of disease; hence the study of the conditions of life of these micro-organisms and the chemical investigation of the products produced by them must be of infinite importance.

If in the autolysis of animal tissues micro-organisms are added and if no antiseptic is present which prevents their development, then they increase abundantly because of the favorable conditions for development. At the same time the enzymes are also formed to a great extent and by whose aid the exchange of matter takes place in the bacteria. It follows that many chemical processes occur depending upon the kind of bacteria present and which are foreign to bacteria-free autolysis. The entire process has been called *putrefaction.* Among the products formed we will mention the sulphureted hydrogen, indol and skatol which chiefly give the odor to putrefying proteins. In regard to other putrefactive products we refer to Chapter VIII. Under ordinary circumstances compounds of a basic nature may also be produced by putrefaction. To this class belong the cadaver alkaloids called *ptomaines,* first found by SELMI in human cadavers and then specially studied by BRIEGER and GAUTIER.[3] Certain of these are poisonous, designated as *toxines,* while others are non-poisonous. They all belong to the aliphatic compounds and generally do not contain oxygen. As an example of these basic substances

[1] Hofmeister's Beiträge, 5, p. 142.

[2] Compt. rend. Soc. Biol., 70, 623 (1911).

[3] Selmi, Sulle ptomaine od alcaloidi cadaverici e loro importanza in tossicologia, Bologna, 1878; Ber. d. d. chem. Gesellsch., 11, Correspond. by H. Schiff; Brieger, Ueber Ptomaine, Parts 1, 2, and 3, Berlin, 1885–1886; A. Gautier, Traité de chimie ppliquée à la physiologie, 2, 1873, and Compt. rend., 94.

we must mention the two diamines, *cadaverine* or pentamethylenediamine, $C_5H_{14}N_2$, and *putrescine* or tetramethylenediamine, $C_4H_{12}N_2$, which have awakened special interest because they occur in the contents of the intestine and in the urine in certain pathological conditions, especially in cholera and cystinuria.[1] The putrefaction bases *marcitine*, $C_8H_{19}N_3$, *putrine*, $C_{11}H_{26}N_2O_3$, and *viridinine*, $C_8H_{12}N_2O_3$, isolated by ACKERMANN, also belong to this group. Of special interest is the bacterial poison isolated by FAUST,[2] called *sepsine*, $C_8H_{14}N_2O_2$, which is the substance producing the characteristic toxic action of putrefactive masses. Sepsine was prepared by FAUST as a crystalline sulphate which, on repeated evaporation of its solution, was readily converted into cadaverine sulphate.

Of especially great interest are the toxines which are found in the higher plants and animals, like the jequirity-bean and castor-seed, in the poison of snakes and spiders, in blood-serum, etc., and particularly those produced by pathogenic micro-organisms have an unmistakable relation to the enzymes. A closer study of these various bodies, lysines, agglutinines, toxines, etc., as well as of the antitoxines and the theory of immunity, does not lie within the scope of this work, but on account of the great importance of the subject it will be briefly discussed on page 66.

Classification of the Enzymes. If we exclude those processes which are the result of several enzymotic reactions (i.e. autolysis, putrefaction) then the most important enzymotic processes studied so far are the following:

1. Hydrolytic cleavage processes.
2. Cleavages of another variety (fermentation).
3. Oxidations.

We have no general chemical reaction in the ordinary sense which is common to all enzymes or ferments and each enzyme is characterized by its action and by the conditions under which this action is developed. As the action of an enzyme upon a substance, or related substances, or groups is limited therefore these substances or groups are called the substrate of the enzyme.

In regard to the terminology it must be remarked that an enzyme is often named after the substrate (amylase, protease, lipase); in other cases the kind of action determines the name (oxidase, reductase) and finally one of the products produced by its action forms the basis for the name (alcoholase).

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[1] See Brieger, Berlin. klin. Wochenschr., 1887; Baumann and Udransky, Zeitschr. f. physiol. Chem., **13** and **15**; Brieger and Stadthagen, Berlin. klin. Wochenschr., 1889.

[2] Faust, Arch. f. exp. Path. u. Pharm., **51**; Ackermann, Zeitschr. f. physiol. Chem., **54** and **57**.

Of the above mentioned enzymotic reactions the hydrolytic cleavage processes have been best studied, and the general properties of the enzymes which will be given apply chiefly to the hydrolytically splitting enzymes. Among these the following are to be mentioned especially:

1. Enzymes which split fats and other esters with the formation of the corresponding alcohol and acid. They are called *lipases* or *esterases.*

2. Enzymes which split complex carbohydrates with the formation of simpler ones. To these belong:

a. Disaccharide splitting enzymes for instance *saccharase* (invertase, invertin), *maltase, lactase* which act upon the corresponding disaccharide saccharose (cane-sugar) maltose and lactose (milk sugar);

b. Polysaccharide splitting enzymes such as *amylase, ptyalin.* The name *diastase* is often used to designate all the enzymes of this group. In close relation to these enzymes stand the *glucoside splitting* enzymes which occur especially in higher plants and the best known of which is *amygdalase* (*emulsin*) occurring in the almond.

3. Enzymes which act upon the proteins or their related cleavage products. Of these we have:

a. Peptidases and *erepsin* which split polypeptides or peptones;

b. Proteases which act upon proteins as substrate (pepsin, trypsin, autolytic enzymes).

Among the hydrolytic enzymes of the animal kingdom we also include the *arginase,* which splits arginine into urea and ornithin and the *histozym,* which splits hippuric acid. The two following groups also belong here, namely, the *nucleases* which split nucleic acids and which will be discussed in Chapter II, and the coagulating enzymes, *rennin* and *thrombin,* which are probably active as proteases. The deamidizing enzymes which split off the NH_2 group from amino combinations are, at least in certain cases, to be classed as hydrolytic enzymes. This is for example the case with the *adenase* and *guanase* which splits off ammonia from the two bodies adenine and guanine converting them into hypoxanthine and xanthine respectively. The *urease* which splits urea also belongs to this group.

General Properties of the Enzymes. When possible we make use of watery solutions of enzymes in experimentation. In case they are insoluble in water (certain lipases) we use them in the form of more or less purified powders or together with the tissue where they are formed. We have no general method for preparing enzyme solutions. In certain cases they are contained in secretions (gastric and pancreatic enzymes); in others they are prepared from the cells by crushing and pressing out the cell juice (zymase, organ enzymes), and finally, most enzymes can be extracted from the cells with water or glycerin, and as this last gives permanent solutions it has found great use as an extraction medium.

The aqueous solutions can be kept at low temperatures for a long time after the addition of toluene or chloroform.

In all these cases the enzymes are obtained strongly contaminated with other bodies, especially by proteins. Only in exceptional cases is it possible to free the enzyme solution from protein so that the solution does not give the ordinary protein reactions. This is true for the solution of saccharase obtained from yeast by treatment with water; if this is shaken with kaolin the protein is adsorbed by the kaolin while the solution contains the enzymes.[1]

No enzyme has thus far been obtained in a perfectly pure form, and the chemical constitution as well as structure is therefore unknown. The enzymes probably belong to the colloids; if they themselves are not colloids, they occur at least with colloids, from which they may be separated only with difficulty, if at all. The enzymes are characterized by the fact that they are readily taken up by finely divided substances (inorganic precipitates, carbon, kaolin, infusorial earth and other colloids such as alumina, iron hydroxide, proteins[2]). This process may act selectively, as from a solution certain enzymes can be taken up and others not at all, or only to a slight extent (HEDIN,[3] MICHAELIS and EHRENREICH[4]). The adsorption process is more or less irreversible and differs in this from the adsorption of crystalloid substances. Still the trypsin and rennin adsorbed by charcoal can be to a slight extent expelled from the charcoal by means of other adsorbable substances such as casein and albumin (HEDIN).[5] Rennin taken up by charcoal can to a very slight extent be set free by the addition of glucose (HEDIN) and saccharase adsorbed by charcoal can be set free by cane-sugar (ERIKSSON).[6] As we will learn below, the adsorbed enzyme is inactive. The so-called shaking inactivation of enzymes or the loss in activity of enzymes, which occurs on shaking their solution seems to be due to an adsorption of the enzyme when it is either taken up by the precipitate formed on shaking (ABDERHALDEN and GUGGENHEIM) or is concentrated at the surface between the solution and the froth (S. AND S. SCHMIDT-NIELSEN).[7] These latter found the inactivation of rennin by shaking was regained if the froth was allowed to subside.

All enzymes lose their specific action on sufficiently heating their

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[1] Michaelis, Bioch. Zeitschr., **7**, 488 (1907).

[2] Dauwe, Hofmeister's Beiträge, **6**, 426 (1905).

[3] Bioch. Journ., **2**, 112 (1907).

[4] Bioch. Zeitschr., **10**, 283 (1908).

[5] Bioch. Journ., **2**, 81 (1906); Zeitschr. f. physiol. Chem., **63**, 143 (1909).

[6] Hedin, *ibid.*, **63**, 143 (1909); Ericksson, *ibid.*, **72**, 313 (1911).

[7] Abderhalden and Guggenheim, Zeitschr. f. physiol. Chem., **54**, 352 (1907); S. and S. Schmidt-Nielsen, *ibid.*, **68**, 317 (1910) which also contains the literature.

aqueous solutions, and even at ordinary temperature the enzymes are gradually decomposed. In general the enzymes lose their activity by heating for a short time to 70° C. MADSEN and WALBUM have followed this process at different temperatures and found that the decomposition of trypsin, pepsin and rennin at given temperatures proceeds monomolecularly, i.e., that the velocity of reaction at every moment is proportional to the concentration of the enzyme (page 34).[1] The readiness with which an enzyme is decomposed is nevertheless to a great extent dependent upon the presence of other bodies (page 54).

Certain enzymes are also sensitive to light. According to SCHMIDT-NIELSEN[2] rennin is injured by light and in particular, by the ultra-violet rays. The experiments of JODLBAUER and TAPPEINER[3] with invertin have led to the same results; the visible rays can also in some cases (peroxidase, hæmase) in the presence of oxygen or certain fluorescent substances exert an injurious action.[4]

According to SCHMIDT-NIELSEN[5] the weakening in the rennin under the influence of light proceeds like a monomolecular reaction.

Experiments on the *cataphoresis* of enzymes have been made by BIERRY, HENRY and SCHAEFFER[6] as well as by MICHAELIS. These investigators found that saccharase migrates to the anode. MICHAELIS[7] found that the migration direction of other enzymes was dependent upon the reaction, namely in faintly acid reaction they moved to the cathode and in faintly alkaline reaction to the anode. Recently PEKELHARING and W. E. RINGER[8] have observed that the migration direction of pig pepsin was very materially influenced by the addition of small amounts of proteoses. From what was previously stated (page 20) the saccharase must therefore have a negative charge. As MICHAELIS,[9] has on the other hand, found that the saccharase can be adsorbed by the positively charged aluminium hydrate and not by the negatively charged kaolin, he concludes that the formation of adsorption compounds, at least in certain cases, depends upon an opposed electric charge of the two components.

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[1] Arrhenius, Immunochemie, Leipzig, 1907, 58.

[2] Hofmeister's Beiträge, **5**, 355 (1904); **8**, 481 (1906); Zeitschr. f. physiol. Chem., **58**, 233 (1908).

[3] Arch. f. klin. Med., **87**, 373 (1906).

[4] Bioch. Zeitschr., **8**, 61 and 84 (1908). See also Agulhon, Compt. rend., **153**, 979 (1911).

[5] Zeitschr. f. physiol. Chem., **58**, 232 (1908).

[6] Compt. rend. soc. biol., **63**, 226 (1907).

[7] Bioch. Zeitschr., **16**, 81, 486; **17**, 231 (1909).

[8] Zeitschr. f. physiol. Chem., **75**, 282 (1911).

[9] Bioch. Zeitchr., **10**, 299 (1908).

Like the colloids the enzymes only diffuse very slowly and the diffusion through membranes does not occur in most cases; only certain membranes such as collodion tubes allow certain enzymes to pass through. The collodion tubes can be impregnated in such a way with lecithin or cholesterin that the diffusion is very slight. The same applies to the filtration through collodion membranes (BIERRY and SCHAEFFER).[1] It must not be forgotten in such experiments that the membrane can adsorb a considerable part of the enzyme (BECHHOLD).[2]

Just as it is difficult to prepare an enzyme free from non-enzymotic contaminations, so also is it difficult to exclude the possibility that a so-called enzyme is not a mixture of several related enzymes. In fact the several enzymotic processes proceed step by step, and it is possible that the various steps are caused by different enzymes. Thus the decomposition of protein into amino-acids, with proteoses, peptones, and polypeptides as intermediary products, may be the result of the activity of several enzymes which are active one after another or are parallel with one another in activity. Erepsin does not attack genuine proteins, but completes the decomposition which has been begun by other enzymes (pepsin, trypsin).

The enzymes are formed within the living cells. In certain cases the cells do not secrete the complete enzyme, but substances which are transformed first outside of the cells into active enzymes. These preliminary steps or mother substances of the enzymes have been called *proenzymes* or *zymogens*. These under certain conditions are changed into enzymes and in certain cases this is brought about by the interaction of special but not well known substances which have been called *kinases* (see Chapters V and VIII). In other cases the transformation of the zymogen into the active enzyme is brought about by well defined chemical substances. Thus the proenzymes of pepsin and of rennin are activated by acids (see below on the retardation of enzyme action and also Chapter VIII).

In certain other cases the presence of bodies which resist temperature and are dialyzable and therefore not enzymes, are necessary or helpful besides the real organic enzyme. Thus the presence of an acid is necessary for the action of pepsin and hydrocyanic acid, according to MENDEL and BLOOD,[3] favors to a high degree the action of papain (a plant protease). R. MAGNUS[4] has been able to separate by dialysis, from a solution of liver-lipase, a body which is necessary for the action upon amyl

[1] Compt. rend. soc. biol., **62**, 723 (1907).
[2] Zeitschr. f. physik. Chem., **60**, 257 (1907).
[3] Journ. of biol. Chem., **8**, 177 (1910).
[4] Zeitschr. f. physiol. Chem., **42**, 149 (1904).

salicylate. Enzymes made inactive by dialysis can be activated again by the addition of boiled enzyme or the concentrated dialysate. HARDEN and YOUNG[1] on filtering yeast-press juice through earthenware filters impregnated with gelatin, have found different constituents of the zymase on the filter and in the filtrate. The true enzyme remains on the filter. This alone is inactive, but becomes active when the other part which has passed through the filter, and which is dialyzable and resistant to temperature, is added. This part is consumed during fermentation and therefore the enzyme becomes inactive. After the addition of, best, boiled press-juice to this the fermentation begins again (see also Chapter III). Certain of the just-mentioned substances which are resistant to heat, whose presence are necessary for the action of certain enzymes, are ordinarily called *co-enzymes*. As they are not to be classified with the enzymes, they are more correctly called *activators*, as suggested by EULER.[2] Their action is probably different in different cases, and differs also from the activating action of the kinases.

Many enzymes are secreted by the cells as such or as proenzymes. They act outside of the cells in which they were formed, or they act after having been transformed into the enzyme, and hence are called *secretion* enzymes or *extracellular* enzymes. Besides these extracellular enzymes we also have another group which acts within the cells, hence are intracellular and therefore are called *intracellular* enzymes or *endo-enzymes*. To this group belongs, beside the yeast zymase, numerous enzymes such as oxidases and hydrolytic enzymes.

Formation and Secretion of Enzymes. The investigations of PAWLOW[3] and his pupils upon the formation and secretion of the enzymes active in the alimentary tract are very important. According to these investigations the amount of secretion of the glands and the behavior of the enzymes contained in the secretion are dependent upon the amount and composition of the food taken and in such a manner that the kinds and amounts of enzymes are appropriate for the digestion of the food-stuffs (see Chapter VIII). Similar results were also obtained by WEINLAND[4] who found that the pancreas does not normally contain any lactase but did contain this enzyme after feeding the animal with milk or milk sugar. This has been substantiated by BAINBRIDGE.[5] Analogous experiments have been made with salivary ptyalin by NEIL-

[1] Proc. Physiol. Soc., **32** (1904); Proc. Chem. Soc., **21**, 189 (1905); Proc. Roy. Soc., **77** (ser. B), 405 (1906); *ibid.*, **78**, 369 (1906).

[2] Zeitschr. f. physiol. Chem., **57**, 92 (1908).

[3] Arbeit der Verdauungsdrüsen, Wiesbaden, **1898, s. 51.**

[4] Zeitschr. f. Biol., **38**, 607 (1899); **40**, 386 (1900).

[5] Journ. of Physiol., **31**, 98 (1904).

SON and LEWIS[1] with the same results. On the other hand the correctness of these observations is disputed by BIERRY,[2] PLIMMER,[3] WOHLGEMUTH[4] and POPIELSKI[5] as they could not find any accommodation. MENDEL[6] and his co-workers by careful investigations on certain enzymes obtained from embryonal intestine and other embryonal tissues could not find any marked difference between these enzymes and the enzymes of the full grown animal. These results speak against the accepted influence of the food and of the processes depending upon the taking up of food, upon the formation of enzymes. Recently the investigations of LONDON[7] and his collaborators upon the influence of the food upon the digestion juices have shown that the amount of juice secreted is dependent upon the constitution of the food but not the ferment content of the same. The observations of COHNHEIM[8] also speak against the view that the kind and quantity of enzymes secreted in the intestinal tract accommodate themselves to the digestion, as he found that the organism secretes as much fluid (gastric juice, pancreatic juice and bile) when already digested food is introduced into the stomach as when undigested food is introduced. ARRHENIUS[9] has calculated from LONDON'S figures, that the total amount of digestive juice secreted was proportional to the quantity of food-stuffs. From experiments which EULER and his collaborators have made upon the formation of inverting enzymes he concludes that we have inverting enzymes whose formation is specific by getting accustomed to the substrate, while the formation of others is in no wise thus influenced.[10]

In this connection we will call attention to the appearance of enzymotic substances in the blood after the subcutaneous or intravenous (parenteral) introduction of certain food-stuffs. WEINLAND first showed that the parenteral introduction of cane-sugar caused the appearance in the serum of a cane-sugar splitting enzyme.[11] ABDERHALDEN and KAPFBERGER[12] have substantiated and developed these observations. Bodies having a similar action also appear after the injection of milk

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[1] Journ. of Biol. Chem., **4**, 501 (1908).
[2] Compt. rend. soc. biol., **58**, 701 (1905).
[3] Journ. of Physiol., **34**, 93 (1906).
[4] Bioch. Zeitschr., **9**, 1 (1908).
[5] Pflüger's Arch., **127**, 443 (1909).
[6] Amer. Journ. of Physiol., **20**, 81, 97 (1907); **21**, 64, 69, 85, 95 (1908).
[7] Zeitschr. f. physiol. Chem., **68**, 366 (1910).
[8] *Ibid.*, **84**, 419 (1913).
[9] *Ibid.*, **63**, 323 (1909), see also London, *ibid.*, **65**, 189 (1910).
[10] *Ibid.*, **70**, 279; **76**, 388; **78**, 246; **79**, 274; **80**, 241 (1912).
[11] Zeitschr. f. Biol., **47**, 279 (1905).
[12] Zeitschr. f. physiol. Chem., **69**, 23 (1910).

sugar and of starch. ABDERHALDEN and his co-workers have shown that the parenteral introduction of protein or peptone gives the blood serum of the animal the power of splitting proteins, which power is destroyed on heating to 60–65° C.[1] The introduction of very large quantities of sugar or proteins per os (over feeding) has the same effect as the parenteral introduction. ABDERHALDEN considers the active substances thus obtained as enzymes. The question is still undecided whether the substances introduced bring about a formation of the enzymes or whether they only transport the already formed enzymes to the blood.

Heat Production. The question whether in the hydrolytic processes with the aid of enzymes heat is given off or taken up has been attacked in two different ways. GRAFE[2] could not find either any setting free or taking up of heat in the digestion of protein in a RUBNER calorimeter. On the other hand HARI[3] by determining the calorific values of albumin before and after digestion came to about the same results. If we exclude the work developed in the process then it follows that the energy supply of the organism is not perceptibly changed by the hydrolytic cleavages of the protein. The chief source of energy is to be sought in the oxidation processes that follow the cleavages.

Modes of Action of the Enzymes. The enzymes do not suffer any appreciable change during the reaction they perform, and insignificant amounts of the enzyme are able to decompose relatively enormous amounts of the substrate. For example, 1 part of saccharase can invert 100,000 parts of cane-sugar (O'SULLIVAN and THOMPSON)[4] and 1 part of rennin can decompose more than 400,000 parts of casein (HAMMARSTEN)[5]. For these reasons the enzymes have for a long time been considered as catalytic substances. Nevertheless the enzyme reactions always take place in heterogeneous media where on one hand the enzyme exists as colloid and on the other the substrate in many cases is a colloid (starch, proteins). As above mentioned, the enzymotic decompositions are often complicated by their taking place over several intermediary steps to the final product. As indicated by several conditions, the enzymes also, before they act upon the substrate, combine therewith in some way or another. The fact that the action of an enzyme is dependent upon the stereometric construction (page 61) of the substrate speaks essentially for this view. The substrate also protects certain enzymes against destruc-

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[1] *Ibid.*, **61**, 200; **62**, 120, 243 (1909); **64**, 100, 423, 426, 427; **66**, 88; **69**, 23 (1910); **71**, 110, 367, 385 (1911). See also **77**, 250 (1912).

[2] Arch. f. Hygiene, **62**, 216 (1907).

[3] Pflüger's Arch., **115**, 11 (1906); **121**, 459 (1908).

[4] Journ. chem. Soc., **57**, 926 (1890).

[5] See Maly's Jahresber, 7.

tive influences (heat, alkalies)[1]; According to this only that part of the added enzyme which is combined with the substrate is active. In judging of the rapidity of enzyme reactions the following must be considered:

1. The velocity with which the enzyme combines with the substrate.
2. The result of the division, i.e., how much of the added enzyme is combined with the substrate.
3. The velocity of the chemical processes produced by the enzyme.

The velocity of the combination of the enzyme with the substrate (1) can at least in many cases be ignored in consideration of the time necessary for the chemical reaction (see page 37). This applies to those cases where the chemical transformation in the presence of an excess of substrate at the beginning of the processes remains the same in each successive time interval. The quantity of enzyme combined with the substrate, does not, in these cases, increase with the time, which would be the case if the time necessary for the combination is not in comparison with that for the chemical reaction. Equal decomposition for equal time at the beginning of the processes have been found for the following enzymes—invertase,[2] diastase,[3] trypsin with casein,[4] as substrate.

The second question, as to the division of the enzyme between different phases we will discuss after we have spoken of the velocity of the real chemical reaction (page 58) as well as the retardation of enzyme action (page 62).

In regard to the chemical reaction they proceed probably in a different manner according to the kind of combination between the substrate and the enzyme. In one case we can consider that the combination of the enzyme with the substrate is of such a kind that both form a homogeneous phase and that one serves as solvent for the other (page 27). In this case the chemical reaction produced by the enzyme takes place in a homogeneous medium. Secondly, we can consider the combination of the substrate and enzyme as an adsorption combination (see page 27) in which case the combination does not form a homogeneous phase and the reaction differs more or less from one taking place in a homogeneous system. Bearing this in mind it would be interesting to investigate whether the facts found for enzymotic reactions correspond with catalytic reactions in homogeneous media.

For these latter the following laws (see page 33) have been found:

1. When the quantity of catalyst remains constant, the reaction

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[1] O'Sullivan and Thompson, Journ. Chem. Soc., **57**, 926 (1890); Bayliss and Starling, Journ. of Physiol., **30**, 71 (1903); Hedin, *ibid.*, **30**, 173 (1903); **32**, 474 (1905); Taylor, Journ. of biol. Chem., **2**, 90 (1906).

[2] O'Sullivan and Thompson, Journ. Chem. Soc., **57**, 926 (1890); Ducleau, Traité de microbiologie II, 137; Brown, Trans. Chem. Soc., **81**, 373 (1902); Armstrong, Proc. Roy. Soc., **73**, 500 (1904); Hudson, Journ. Amer. Chem. Soc., **30**, 1160, 1564 (1908).

[3] Brown and Gliddinning, Proc. Chem. Soc., **18**, 43 (1902).

[4] Hedin, Journ. of Physiol., **32**, 471 (1905).

velocity for every moment is proportional to the concentration of the body decomposed, which is shown by the velocity coefficient in the same experiment being constant at different times.

2. The velocity coefficient or the reaction velocity with constant concentration of substrate is proportional to the quantity of catalyst.

The first law has been shown for certain enzymes in case an excess of enzyme is present, namely for saccharase,[1] lactase[2] and trypsin.[3] It was found that the decomposition in a certain time was proportional to the substrate. In other cases the determination of the correctness of the law was accomplished with difficulty. A part of the enzyme may during an experiment be either destroyed or in other ways (combining with the product) be put out of action; then reverse reactions may take place (page 11) and finally in many cases our analytical methods are incapable of obtaining comparative results for different decompositions, as the reaction in many cases takes place step by step, or several reactions occur at the same time.[4] Only in a few cases with especially simple reactions have constant values been found for the velocity coefficient at the beginning, as long as the quantity of reaction product was small and the active quantity of enzyme remained unchanged according to the formula (see page 11).[5]

$$k=\frac{1}{t}\log\frac{C}{C-x}.$$

Recently HUDSON[6] has found constant values for k for the entire process of inversion of cane-sugar by saccharase in a faintly acid reaction. The reason for the different results of earlier investigators[7] is due, in part, according to HUDSON, to the fact that the multirotation of the glucose formed was not considered by these experimenters before the extent of inversion was determined polariscopically. In the cleavage of salicin by emulsin HUDSON and PAINE[8] obtained constant values for k in the entire process.

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[1] Brown, Proc. Chem. Soc., **18**, 14 (1902).

[2] Armstrong, Proc. Roy. Soc., **73**, 500 (1904).

[3] Hedin, Journ. of Physiol., **32**, 475 (1905).

[4] Hedin, Zeitschr. f. physiol. Chem., **57**, 468 (1908).

[5] Senter, Zeitschr. f. physik. Chem., **44**, 257 (1903); Issajew, *ibid.*, **42**, 102; **44**, 546; Euler, Hofmeister's Beiträge, **7**, 1 (1906); Dietz, Zeitschr. f. physiol. Chem., **52**, 301 (1907); Taylor, Journ. of biol. Chem., **2**, 93 (1906); Nicloux, Compt. rend. soc. biol., **56**, 840 (1904); Rona, Bioch. Zeitschr., **33**, 413 (1911); **39**, 21 (1912); Euler, Zeitschr. f. physiol. Chem., **51**, 213 (1907).

[6] Journ. Amer. Chem. Soc., **30**, 1160, 1564 (1908).

[7] See Henri, Zeitschr. f. physik. Chem., **39**, 194 (1901) also A. J. Brown, Trans. Chem. Soc., **81**, 373 (1902).

[8] Journ. Amer. Chem. Soc., **31**, 1242 (1909).

The second law for catalytic reactions which we have formulated, that with constant quantities of substrate the reaction velocity is proportional to the quantity of enzyme, has been shown in certain cases where the substrate was in excess (practically constant quantity) namely with kephir lactase,[1] trypsin with casein as substrate.[2] In the just-mentioned monomolecular enzyme reactions the velocity coefficient in a few cases was found proportional to the quantity of enzyme (catalase from blood,[3] erepsin with glycyl-glycine as substrate,[4] pancreatic lipase [5]) and in others not (catalase from Boletus scaber, lipase from pig fat).[6] It has been shown for several enzymotic reactions that with the same substrate the same decomposition can be obtained if the time of action varies in inverse proportion to the added quantity of enzyme. If p is the quantity of enzyme and t the time of action, then the decomposition is the same in all tests where $p.t$ is the same figure. This rule has been found true for the following enzymes: saccharase (O'SULLIVAN and THOMPSON as well as HUDSON [7]), pepsin (SJÖQVIST [8]), rennin (especially FULD [9]), peptone-splitting enzyme (VERNON [10]), fibrin ferment of snake poison (MARTIN [11]), trypsin (HEDIN [12]), pepsin, rennin, trypsin, pyocyaneus protease (MADSEN [13]). On the action of trypsin upon casein this law has been shown correct for different stages in the reaction.[14] This indicates that the progress of the entire reaction remains the same with different quantities of enzyme, only that the time for the same decomposition is inversely as the quantity of enzyme. As clearly shown by HEDIN, this indicates that the velocity coefficient is proportional to the quantity of enzyme which is called for by the second law. If we start with the above-mentioned assumption that only that enzyme is active which is combined, then it follows from the proportionality between the velocity coefficient and the quantity of enzyme, that always the same fraction of the enzyme is combined with the substrate, or that the division of the enzyme remains independent of the quantity.

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[1] Armstrong, Proc. Roy. Soc., **73**, 500 (1904).
[2] Hedin, Journ. of Physiol., **32**, 471 (1905).
[3] Senter, Zeitschr. f. physik. Chem., **44**, 257 (1903).
[4] Euler, Zeitschr. f. physiol. Chem., **51**, 213 (1907).
[5] Kastle and Loevenhart, Amer. Chem. Journ., **24**, 491 (1900).
[6] Euler, Hofmeister's Beiträge, **7**, 1 (1906).
[7] Trans. Chem. Soc., **57**, 926, 1890; Journ. Amer. Chem. Soc., **30**, 1160, 1564 (1908).
[8] Skand. Arch. f. Physiol., **5**, 358 (1895).
[9] Hofmesietr's Beiträge, **2**, 169 (1902).
[10] Journ. of Physiol., **30**, 334 (1903).
[11] *Ibid.*, **32**, 207 (1905).
[12] *Ibid.*, **32**, 468 (1905); **34**, 370 (1906).
[13] Arrhenius, Immunochemie, Leipzig, 1907, 46.
[14] Zeitschr. f. physiol. Chem., **57**, 478 (1908).

In determining the quantity of enzyme the so-called SCHÜTZ's rule plays an important part. In its newest form this is, that the decomposition is proportional to the square root of the quantity of enzyme and the time, or decomposition $= k\sqrt{pt}$ where k is a constant, p the quantity of enzyme and t the time of the action. This was first shown by SCHÜTZ [1] for pepsin and also, in this form, decomposition $= k\sqrt{p}$ as the time (t) was constant. The form decomposition $= k\sqrt{pt}$ was given by SCHÜTZ, and HUPPERT.[2] According to PAWLOW this rule also applies to trypsin digestion.[3] SCHÜTZ's rule is good for a certain stage of digestion only and it indicates that the extent of the validity must be very dependent upon the method used for the determination of the decomposition as the different digestion products are determined by different methods. It must also be remarked that within the entire domain where SCHÜTZ's rule is applicable the same value for pt must correspond to the same decomposition, and necessarily the above-discussed enzyme-time rule must also be valid. SCHÜTZ's rule has also been proved for the action of gastric and pancreatic lipase.[4] According to ARRHENIUS [5] the validity of the rule can be explained by the assumption that the enzyme combines with the reaction products so that the active mass of enzyme changes in inverse proportion to the quantity of reaction products.

Reversibility of Enzyme Action and Enzymotic Syntheses. Many catalytic processes have been shown to be reversible, i.e., the same catalyst can influence the reaction in different directions according to the concentration of the substances present. Thus far we have only spoken of enzymotic *cleavages;* according to the above it is to be expected that *synthetical* processes can also be produced by enzymes.

The first example of such a reaction was given by CROFT-HILL.[6] He treated a 40 per cent glucose solution with maltase at 30° C. for a very long time and concluded from the change in rotation and reducing power that some maltose was formed from the glucose. EMMERLING [7] showed afterward that a synthesis of maltose did not occur, but rather an isomeric carbohydrate, isomaltose was formed, which is not split by maltase. According to ARMSTRONG [8] emulsin splits isomaltose, but not maltose, and therefore it can synthesize maltose from glucose. A similar reaction had previously been shown by E. FISCHER and ARMSTRONG,[9] that kefir-lactase produced isolactose and not lactose from galactose and glucose. According to CREMER [10] yeast-press juice has the power of forming glycogen from glucose or fructose.

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[1] Zeitschr. f. physiol. Chem., **9**, 577 (1885).

[2] Pflüger's Arch., **80**, 470 (1900).

[3] Arbeit der Verdauungsdrüsen, Wiesbaden, 1898, 33.

[4] Stade, Hofmeister's Beiträge, **3**, 318 (1903); Engel, *ibid.*, **7**, 77 (1906), see Fromme, *ibid.*, **7**, 77, (1906).

[5] Immunochemie, 1907, 43.

[6] Journ. of chem. Soc., **73**, 634 (1898).

[7] Ber. d. d. chem. Gesellsch., **34**, 600 and 2207 (1901).

[8] Proc. Roy. Soc. (ser. B), **76**, 592, (1905)

[9] Ber. d. d. chem. Gesellsch., **35**, 3151, (1902).

[10] *Ibid.*, **32**, 2062 (1899).

A. DANILEWSKI first made the observation that concentrated solutions of peptic cleavage products of protein substances separates an insoluble substance under the influence of rennin. This phenomenon has since been observed by various investigators and the precipitate has been called *plastein* by SAWJALOW [1] and *coagulose* by LAWROW.[2] The same phenomenon is also obtained by other proteolytic enzymes.[3] The plasteins are considered by various investigators as synthetically formed protein. The best proof for this view has been given by HENRIQUES and GJALBÄCK. They show with the formol titration (see Chapter II) that the nitrogen titratable by formol diminishes in the reaction and they also found that the nitrogen precipitatable by tannic acid was increased in the plastein formation. In a later work these authors find that peptic cleavage products from proteins show a plastein formation when under the influence of pepsin-hydrochloric acid in concentrated solution while in dilute solution the cleavage goes further and they conclude from this that the process is reversible. Even protein which has been partly split by acid or alkali shows a plastein formation with pepsin-hydrochloric acid.[4]

The behavior of amygdalin and its cleavage products with enzymes requires special mention. The cleavage takes place step by step as follows:

$$\underset{\text{Amygdalin}}{C_{20}H_{27}NO_{11}} + H_2O = \underset{\text{Mandelic acid nitrileglucoside}}{C_6H_5.CH(OC_6H_{11}O_5).CN} + \underset{\text{Glucose}}{C_6H_{12}O_6}. \quad (1)$$

$$\underset{\text{Mandelic acid nitrileglucoside}}{C_6H_5.CH(OC_6H_{11}O_5).CN} + H_2O = \underset{\text{Mandelic acid nitrile}}{C_6H_5.CH\begin{matrix}CN\\OH\end{matrix}} + \underset{\text{Glucose}}{C_6H_{12}O_6}. \quad (2)$$

$$\underset{\text{Mandelic acid nitrile}}{C_6H_5\overset{*}{C}H\begin{matrix}CN\\OH\end{matrix}} = \underset{\text{Benzaldehyde}}{C_6H_5C\begin{matrix}O\\H\end{matrix}} + \underset{\text{Hydrocyanic acid}}{HCN}. \quad (3)$$

The entire process with the formation of the end products sugar, benzaldehyde and hydrocyanic acid takes place under the influence of emulsin from almonds. The first part of the process can be especially brought about by the influence of yeast (FISCHER)[5] and the second and third parts under the influence of prunase from the leaves of Prunceae.[6] Of

[1] Zeitschr. f. physiol. Chem., **54**, 119 (1907).
[2] *Ibid.*, **51**, 1; **53**, 1 (1907); **56**, 343 (1908); **60**, 520 (1909).
[3] Kurajeff, Hofmeister's Beiträge, **4**, 476 (1904); Nürnberg, *ibid.*, **4**, 543 (1904).
[4] Zeitschr. f. physiol. Chem., **71**, 485 (1911); **81**, 439 (1912).
[5] Ber. d. d. chem. Gesellsch., **28**, 1508 (1896).
[6] H. E. Armstrong, E. F. Armstrong and Horton, Proc. Roy. Soc., **85**, 359, 363, 370 (1912).

the three above given reactions 1 and 3 can be reversed by enzymes and indeed 1 even by using yeast (EMMERLING)[1] and 3 with emulsin (ROSENTHALER[2]). In the last instance the reaction is asymmetric in that the *d*-form of the mandelic acid nitrile is formed. The asymmetric C atom is marked in the above formula. Subsequently ROSENTHALER was able to divide the emulsin into a splitting component (*δ*-emulsin) and a synthetical form (*σ*-emulsin)[3]. In connection with the views on the structure and mode of action of enzymes it is of special interest that recently BREDIG and FISKE[4] have been able to prepare the two optical antipodes of mandelic acid nitrile from benzaldehyde and hydrocyanic acid by means of optically active catalysts. By using quinine as catalyst the dextro-rotatory nitrile was formed and by quinidine (isomeric with quinine but opposed in regard to rotation power) the lævorotatory nitrile was formed. This indicates that possibly the enzymes also have an asymmetric structure. The synthetic formation of glucosides by the aid of emulsin has also been performed by VAN'T HOFF.[5]

An undoubted synthesis of fat and other ester-like combinations of fatty acids is also known. KASTLE and LOEVENHART[6] have shown the formation of ethyl butyrate from ethyl alcohol and butyric acid under the influence of a pancreas enzyme. In an analogous manner HANRIOT[7] obtained monobutyrin from butyric acid and glycerin with blood serum. POTTEVIN[8] by means of a pancreas enzyme transformed oleic acid and glycerin into mono- and triolein as well as oleic acid esters with monatomic alcohols. The synthetical action of the pancreas has been closely studied by DIETZ.[9]

The enzyme used by DIETZ was insoluble in water, and its action was tested with *i*-amyl alcohol and *n*-butyric acid or the corresponding ester. It was shown that the reaction took place in the insoluble phase (enzyme). From the formula alcohol+acid$\rightleftarrows$ester+water, it follows that when the molecular concentrations of alcohol, acid, ester and water are designated C_A, C_S, C_E, C_W, the reaction velocity of the ester formation for a homogeneous system is $\frac{dx}{dt}=k_1.C_A.C_S-k_2.C_E.C_W$ (see page 32), which equation can be simplified to $\frac{dx}{dt}=k_1.C_S-k_2.C_E$ as the alcohol and water were in excess and their concentration considered as constant and included in the constants k_1 and k_2. At equilibrium we have $k_1C_S=k_2C_E$ or

[1] Ber. d. d. chem. Gesellsch., **34**, 3810 (1901).
[2] Bioch. Zeitschr., **14**, 238 (1908).
[3] *Ibid.*, **17**, 257 (1909).
[4] Bioch. Zeitchr., **46**, 7 (1912).
[5] Sitzungsber. preuss. Akad. Wiss., 1909, s. 1065; 1910 s. 963.
[6] Amer. Chem. Journ., **24**, 491 (1900).
[7] Compt. rend., **132**, 212 (1901).
[8] *Ibid.*, **136**, 1152 (1903), **138**, 378 (1903); Ann. Inst. Past., **20**, 901 (1906).
[9] Zeitschr. f. physiol., Chem., **52**, 279 (1907).

$\frac{k_1}{k_2}=\frac{C_E}{C_S}=K$ (page 32). It follows that the same equilibrium is attained irrespective of whether we start with alcohol+acid, or ester+H_2O. The equilibrium is also independent of the antecedents as well as the quantity of enzyme.

On comparing the equilibrium constants (K) which are obtained with different quantities of ester or acid, it is shown that in the above equation $\sqrt{C_E}$ must be introduced instead of C_E in order to obtain constant values for K. In the saponification of the ester the reaction velocity is proportional to $\sqrt{C_E}$, and not C_E. According to DIETZ this is due to the fact that the system is a heterogeneous one, and that only that part of the ester which is absorbed by the solid phase (enzyme) takes part in the reaction. The velocity constant of the ester formation is shown to be proportional to the quantity of enzyme.

According to what was stated above (page 35), the equilibrium in a reversible reaction must be independent of the nature of the catalyst. This was not the case in DIETZ's experiments. With picric acid as the catalyst a different equilibrium was obtained than with the pancreas enzyme. With the acid as catalyst the equilibrium was moved toward the direction of the ester. While this action is not understood it may perhaps be explained by the fact that the system in one case was homogeneous and in the other case heterogeneous.

Similar observations that the enzymotic end-condition can be different from the stabile end-condition of the same system have previously been made by TAMMANN,[1] but in these cases generally so-called false equilibrium existed, which for example, by the addition of more enzyme changed, so that the cleavage proceeds further. These false equilibria are generally caused by the enzyme being destroyed or put out of action in other ways.

Among the enzymotic-ester syntheses we must also include the formation of carbohydrate phosphoric acid ester in fermenting sugar solutions in the presence of soluble phosphates, as first observed by HARDEN and YOUNG.[2] These will be discussed in detail in Chapter III.

It is seen that enzymotic syntheses are known. From this it follows that the questionable enzyme reactions are to be considered as reversible. In certain cases another substance which cannot be split by the enzyme is formed while in other cases the opposite direction of the reaction can be detected by means of various constituents of the same enzyme solution.

Specificity of Enzyme Action. It has been known for a long time that a great difference exists in regard to the action of enzymes in the sense that different enzymes act only upon certain classes of bodies (proteins, carbohydrates, fats). Then there also exist differences in the manner in which different enzymes of the same group influence different members of the same class (maltase, lactase, saccharase). Finally, it is possible for one enzyme to attack one of two optical antipodes and the other not at all, or only to a slight degree. That optical antipodes are burned with unequal facility in the organism was shown by E. FISCHER, and that of the numerous aldohexoses only three, *d*-glucose, *d*-mannose

[1] Zeitschr. f. physiol. Chem., 16, 271 (1892).

[2] Proc. Roy. Soc. B, 1908 p. 209.

and *d*-galactose, and of the ketohexoses only one, *d*-fructose are fermentable; and then that the synthetically prepared stereoisomeric glucosides behave differently with the enzymes. Thus of two isomeric glucosides, one methyl-*d*-glucoside, the (α) was attacked by yeast and the other (β) only by emulsin, while the corresponding methyl-*l*-glucosides were not split by either of these enzymes. The corresponding glucoside obtained from galactose behaves in a similar manner.[1] On the behavior of amygdalin to various enzymes see page 59. In connection with these observations FISCHER presents the theory that for the action of an enzyme a certain correspondence in stereometric structure of the enzyme and substrate must exist; the enzyme must fit the substrate somewhat like a key fitting a lock.

Then followed similar observations of DAKIN,[2] who found that racemic mandelic acid ester, on incomplete hydrolysis by liver press-juice, yielded a strongly dextrorotatory acid, while the ester remaining was levorotatory. The dextrorotatory ester was more quickly hydrolyzed than the levorotatory ester. Finally, we must mention the investigations of FISCHER and ABDERHALDEN[3] on the cleavage of polypeptides by pancreas press-juice. From abundant material they concluded that those polypeptides which consist entirely of the optical forms of amino-acids occurring in nature are hydrolyzed and the others not. If in a racemic form besides a polypeptide consisting of natural amino-acids, another occurs also, then only the first is hydrolyzed. Besides this, other factors are also of importance. Thus *l*-leucyl-glycine is not hydrolyzed, although both constituents occur in nature. The size of the molecule seems also to be of importance, as mono-, di- and triglycyl-glycine are not split, while tetraglycyl-glycine is. See also Chapter VIII.

Retardation of Enzyme Action. There are several reasons for the assumption that the hydrolytic enzymes are only active after they have combined with the substrate. From this it follows that those substances which prevent the formation of such combination may cause the retardation of enzyme action. For this reason the enzyme action is retarded by such substances which adsorb the enzyme (page 49). HEDIN[4] has made experiments on the retarding action of charcoal upon the action of trypsin upon casein, and the action of rennin upon milk and it was shown that the retardation was more pronounced if the powder and enzyme were allowed to act upon each other before the substrate was added than if this was present from the beginning. This fact indicates

[1] Zeitschr. f. physiol. Chem., **26**, 60 (1898) (collection of Fischer's works).

[2] Journ. of Physiol., **30**, 253 (1903); **32**, 199 (1905).

[3] Zeitschr. f. physiol. Chem., **46**, 52 (1905); **51**, 264 (1907).

[4] Bioch. Journ., **1**, 484; **2**, 81 (1906); Zeitschr. f. physiol. Chem., **50**, 497 (1907); **60**, 143 (1909). See also Jahnson-Blohm, *ibid.*, **82**, 178 (1912).

that the adsorption process is only reversible with great difficulty or that the enzyme to a certain extent is fastened to the charcoal. That the substrate influences the formation of adsorption combination is shown by the fact that the substrate is also adsorbed by the charcoal. A small part of the adsorbed enzyme can indeed be subsequently displaced on the charcoal by other adsorbable substances and in this way become active again. As various substrates are unequally adsorbed by charcoal the retardation is, therefore, also different in degree. The retardation of the saccharase action by charcoal is the same as for the retardation of the trypsin or rennin action (ERIKSSON [1]).

The action of several enzymes is retarded by normal serum. This was first observed by HAMMARSTEN and RÖDÉN [2] for the action of rennin. Besides this certain constituents of the serum as well as other protein containing fluids have a retarding action and in many such cases the order of the addition of the bodies is important. The retardation by charcoal corresponds to this retardation in several ways and this has led HEDIN [3] to the assumption that the retardation in both cases is brought about by a colloidal reaction (adsorption) between the enzyme and a solid or colloid phase. The facts correspond to this assumption namely that during the action of the retarding substance upon the enzyme the amount of water present is without importance for the final result of retardation. Such a retardation by normal serum or fluids containing protein has been observed in the following cases: retardation of trypsin digestion of casein by native seralbumin,[4] retardation of the action of rennin by neutral serum and by white of egg,[5] and the action of saccharase by serum.[6] Besides this HEDIN [7] found a similar retardation by seralbumin upon the digestion of casein by means of the α-protease of the spleen. The retardation by normal serum or seralbumin has been shown in the cases investigated not to be a specific kind, i.e., a given enzyme is retarded about to the same extent regardless from what species of animal it was prepared.

A specific retardation due to kind have been observed in the following cases:

1. The antienzyme obtained by immunization (see page 66) retards in those cases tested, only or chiefly the enzyme used in the immuniza-

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[1] *Ibid.*, **72**, 313 (1911).

[2] Upsala läkareför. förh., **22**, 546 (1887).

[3] Bioch. Journ., **1**, 484 (1906); Zeitschr. f. physiol. Chem., **60**, 364 (1909); Ergebn. d. Physiol., **9**, 433 (1910).

[4] Journ. of Physiol., **32**, 390 (1905); Bioch. Journ., **1**, 474 (1906).

[5] Zeitschr. f. physiol., **60**, 85, 364; **63**, 143 (1909).

[6] *Ibid.*, **72**, 313 (1911).

[7] Hammarsten's Festschr., 1906.

tion. HILDEBRANDT [1] first produced an anti-enzyme toward emulsin; and MORGENROTH [2] obtained in a similar manner an anti-rennin in goats' serum; BORDET and GENGOU [3] immunized against fibrin ferment, SACHS' [4] against pepsin, SCHÜTZE as well as BERTARELLI [5] against various plant lipases, SCHÜTZE [6] against lactase, PRETI as well as SCHÜTZE and BRAUN [7] against diastase, K. MEYER [8] against the proteases of bacillus prodigiosus and bacillus pyocyaneus.

2. The retarding body of the rennin enzyme which was obtained by treating a neutral infusion of the mucous membrane with dilute ammonia and neutralizing, has been recently shown by HEDIN [9] to chiefly retard the enzyme of the same species (see Chapter VIII). In these cases the importance of the order of treatment was also evident.

Most of the retarding substances contained in the serum lose their retarding power on sufficiently heating them. This also occurs in certain cases by treatment with acid. Thus normal horse serum as well as egg-white lose their ability to retard rennin by treatment with very dilute hydrochloric acid and for this reason rennin which has been inactivated by serum or egg-white can be set free again by the use of hydrochloric acid (HEDIN) [10]. Native seralbumin loses its power of attaching itself to trypsin by treatment with dilute acetic acid.

Certain proteins which are digested with difficulty retard the digestion of more readily digestible ones without the order-phenomenon being observed. In such cases the total digestion is probably diminished because the more difficultly digested protein as substrate attracts a part of the enzyme. As the order-phenomenon does not exist, the enzyme is taken up in a complete and readily reversible manner (*enzyme deviation* HEDIN).[11] It is easily understood that the retardation must be less effective than in those cases where the enzyme is attached to the retarding substance. The tryptic digestion of casein in the presence of seralbumin, treated with acid, is diminished by enzyme deviation as well as the digestion of readily split proteins is retarded by egg-white

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[1] Virchow's Arch., **131**, 33 (1893).
[2] Centralbl. f. Bakt., **26**, 349 (1899); **27**, 357 (1900).
[3] Ann. inst. Past. **15**, 129 (1901).
[4] Fortschr. d. Med., **20**, 593 (1901).
[5] Deutsch. med. Wochenschr., 1904; Centralbl. f. Bakt., **40**, 231 (1905).
[6] Zeitschr. f. Hyg., **48**, 457 (1904).
[7] Bioch. Zeitschr., **4**, 6 (1907); Zeitschr. exp. Pathol. u. Therap., **6**, 307 (1909).
[8] Bioch. Zeitschr., **32**, 280 (1911).
[9] Zeitschr. f. physiol. Chem., **72**, 187; **74**, 242; **76**, 355 (1911).
[10] Zeitschr. f. physiol. Chem., **60**, 85, 364 (1909).
[11] *Ibid.*, **52**, 412 (1907).

which is difficult to digest (DELEZENNE and POZERSKI,[1] VERNON,[2] GOMPEL and HENRI,[3] HEDIN [4]).

At this time we must also mention the retarding action which the proteolytic primary cleavage products (proteoses, peptones) exert upon digestion. These products are further split; a part of the enzyme is combined with the products and in this way prevented from dissolving new protein (HEDIN).[5] The retarding power of proteoses and peptones upon rennin action is probably similar to the above.[6]

Finally, the end products of enzymotic activity i.e., bodies which cannot be further split by the enzyme, have also a retarding action on the enzyme action. That the inversion of cane-sugar is retarded by invert sugar has been claimed by many (HENRI,[7] A. J. BROWN,[8] BARENDRECHT,[9] ARMSTRONG[10]), and indeed BARENDRECHT claims that glucose as well as fructose has a retarding action, and that galactose has an even stronger retarding action than the direct cleavage products of cane-sugar. H. E. and E. F. ARMSTONNG[11] found that saccharase, maltase and lactase are retarded by just those varieties of sugar which are produced by their activity. The accumulation of the amylolytic cleavage products have according to SH. LEA,[12] a retarding action upon saliva.

The retarding action of amino-acids upon the decomposition of glycyl-*l*-tyrosine by yeast-press juice has recently been studied by ABDERHALDEN and GIGON.[13] They found that cleavage of peptides is retarded by those optically active amino-acids which occur in the proteins. This result is remarkable in consideration of the observations of FISCHER and ABDERHALDEN that only those polypeptides were split by pancreatic juice which are composed of natural optically active amino-acids (page 62).

The retardation of the action of papain by egg protein and by serum, which is prevented by heating or action of hydrochloric acid, as shown by the investigations of DELEZENNE, MOUTON and POZERSKI as well as by JONESCU and SACHS [14] is a peculiar behavior.

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[1] Compt. rend. soc. biol., **55**, 935 (1603).
[2] Journ. of Physiol., **31**, 495 (1904).
[3] Compt. rend. soc. biol., **58**, 457 (1906).
[4] Zeitschr. f. physiol. Chem., **52**, 422 (1907).
[5] Zeitschr. f. physiol. Chem., **52**, 422 (1907).
[6] *Ibid.*, **46**, 307.
[7] Zeitschr. f. physik. Chem., **39**, 194 (1901).
[8] Journ. Chem. Soc., **81**, 382 (1902).
[9] Zeitschr. f. physik. Chem., **49**, 456 (1904).
[10] Proc. Roy. Soc. (ser. B), **73**, 516 (1904).
[11] *Ibid.*, **79**, 360 (1907).
[12] Journ. of Physiol., 1911.
[13] Zeitschr. f. physiol. Chem., **53**, 251 (1907).
[14] Delezenne, Mouton and Pozerski, Compt. rend., 142; Jonescu, Bioch. Zeitschr., **2**; Sachs, Zeitschr. f. physiol. Chem., **51**, 488 (1907).

In consideration of what has been said (page 58) about enzymotic syntheses it seems very possible in the retardation of enzymotic cleavages by means of cleavage products that we are dealing with synthetic processes where the cleavage products supply the material. This is especially shown by the above-mentioned investigations of ROSENTHALER on emulsin that the retarding action of benzaldehyde or of hydrocyanic acid upon emulsin action, as shown by TAMMANN,[1] is explainable by syntheses. LICHWITZ[2] considers the interaction of the products as a reversible paralyzation of the enzyme.

Appendix: Antigens and Anti-bodies. In connection with the retardation of enzyme action we can also call attention to other similar processes. Under the name *antigen* we include those substances which, when injected into animals, cause the formation of bodies in the organism with which they can in some way or another react. The process is called *immunization* and the bodies formed are called *anti-bodies* or in certain cases *immune bodies*. Generally these anti-bodies are specific in the sense that they only react with the corresponding antigen. The chemical constitution of the antigen as well as of the anti-body is not known; they belong perhaps to the colloids, or at least they occur associated with colloids.

The antigens are either substances soluble in water or occur as constituents of the cells. We will first discuss the antigens soluble in water.

To this group belong, in the first place, certain poisonous substances of animal or plant origin (toxins), for example, snake poisons, bacterial poisons, ricin (from the seeds of *Ricinus communis*), also enzymes as well as certain proteins without special action. The reaction with the anti-bodies (which are obtained in the blood serum of animals) manifests itself with the poisons by the suppression of the poisonous action, with the enzymes by retardation of the enzyme action, and with certain proteins by formation of a precipitate which contains the antigen as well as the anti-body. Anti-bodies of this last type are called *precipitins*.

The longest known (due to the epoch-making investigations of v. BEHRING[3]) and best studied are those anti-bodies which are produced by toxins and which neutralize the action of the toxins upon the animal organism (antitoxins). According to the older view this takes place by some sort of an action of the anti-body upon the cells sensitive to the toxins. After it was shown that the toxins could also be neutralized *in vitro* by the anti-bodies, it is now generally accepted that the neu-

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[1] *Ibid.*, **16**, 271 (1892).
[2] *Ibid.*, **78**, 128 (1912).
[3] Deutsch. med. Wochenschr., 1892; Zeitschr. f. Hygiene, 12 (1892).

tralization is brought about by some sort of a combination between the toxin and the anti-body. The views are very contradictory in regard to the nature of this combination and the manner in which it is formed.

The oldest theory, which has contributed much to our knowledge of these conditions, is that of P. EHRLICH, whom we must thank for the method of measuring the quantity of toxin by injection into an animal. The quantity of toxin which is just sufficient to kill a guinea-pig of given weight in a certain time is selected as the unit. According to the so-called *side-chain theory* of EHRLICH [1] the toxins firstly have a so-called haptophore group, by means of which the toxin can attach itself to a certain cell, and secondly, a so-called toxophore group, by which the toxin exerts its poisonous action. The formation of anti-body after the injection of the toxins EHRLICH explains by the fact that those cells which are attacked by the toxins are supplied with so-called *receptors*, which just fit the haptophore group of the toxins; the toxins are thus anchored on the questionable cells and can then begin their action by aid of the toxophore group. By the attachment of the receptors, the cells are induced to produce new receptors, and indeed, so many receptors are produced that they are thrown off and appear free in the blood plasma. The receptors circulating in the blood are the anti-bodies. As these are able to combine with the toxins they can protect against the toxin those cells which are supplied with the same receptor under whose influence they were found. The toxophore group of the toxins can gradually be destroyed on keeping. A toxin so changed can be continuously anchored to cell-receptors and in this way form anti-bodies, but cannot produce any poisonous action. A toxin without toxophore groups is called a *toxoid* by EHRLICH. It follows that the toxoids can combine with the anti-bodies.

According to EHRLICH, on the neutralization of a toxin a chemical combination takes place between the toxin and the anti-body, and so much of this combination is formed that either the toxin or the anti-body is completely consumed. Now the bacterial poisons are not simple bodies, but mixtures of several poisons of different toxicity and different avidity toward the anti-bodies. Generally the most poisonous is first neutralized, but it also occurs that a less poisonous or indeed a non-poisonous body is first combined with the anti-body (proto-toxoids) or that non-poisonous bodies are combined parallel with the true toxins (syntoxoids). The less poisonous or non-toxic bodies first combined after the binding of the true toxins are called *toxons* (also epitoxoids). According to the relative quantity and the avidity of the different constituents of the toxic solution, the addition of a certain quantity of anti-body can produce entirely different results.

[1] See Michaelis, Die Bindungsgesetze von Toxin und Antitoxin, Berlin, 1905.

ARRHENIUS opposes EHRLICH'S theory that the combination between toxin and anti-body is of a chemical nature, but claims, that their formation does not proceed until one of the components has been used up. An equilibrium is established between the free toxin and the free anti-body on one side and the combination of the two on the other, which the law of mass action requires according to the formula:

$$C_{\text{toxin}} \cdot C_{\text{anti-body}} = K \cdot C^{N}_{\text{toxin + anti-body}} \qquad \text{(page 32)}.$$

For tetanolysin (a substance obtained from tetanus cultures, which dissolves red-blood corpuscles) and its anti-body, as well as for diphtheria toxin and the corresponding anti-body, $n = 2$ was found, i.e., in the combination of a molecule of toxin with a molecule anti-body two molecules toxin-antitoxin combination was formed.

The toxic action which a mixture of toxin and anti-body exerts depends upon the quantity of toxin which, according to the above formula, must always remain free.[1] According to this theory the toxin is a unit poison, as ARRHENIUS[2] now admits with EHRLICH, that the poison is gradually transformed into a non-toxic or only slightly toxic substance which has the same ability to combine with antitoxin as the toxin itself.

EHRLICH'S theory, as well as that of ARRHENIUS admits of a chemical combination between the antigen and the anti-body. According to EHRLICH besides this the substrate (or the cells sensitive to the antigen) combines with the antigen, which is not conformable with the theory of ARRHENIUS.

The combination toxin-anti-body is first gradually produced, and then it is taken up from all sides so that the toxin is fastened to the anti-body by a secondary process (exception, cobra poison). The combination toxin-antitoxin is not reversible in the ordinary sense. This is most easily shown by the fact that to a certain limit more toxin is neutralized according to the time allowed to elapse before the quantity of toxin remaining free is determined by injection into an animal or in other ways.[3] In certain cases it is possible to obtain the toxin again in an active form from the toxin-antitoxin combination, and indeed by treatment with very dilute hydrochloric acid (MORGENROTH[4]). See also page 64 on the setting free of rennin from its combination with normal serum and with egg-white. HEDIN[5] has also been able to obtain

[1] Zeitschr. f. physik. Chem., **44**, 7 (1903).

[2] Immunochemie, Leipzig, 1907, 132.

[3] Martin and Cherry, Proc. Roy. Soc., 1898, 420.

[4] Berl. klin. Wochenschr., 1905, No. 5; Festschr. z. Eröffnung d. pathol. Instit. Berlin, 1906; Virchow's Arch., **190**, 371 (1907).

[5] Zeitschr. f. physiol. Chem., **77**, 229 (1912).

the rennin again in an active form, from the combination of the rennin with anti-rennin obtained by immunization by treatment with hydrochloric acid and then neutralizing.

Recently a third manner of considering the toxin-antitoxin reaction has been presented which is based on the fact that the reaction takes place in a heterogeneous system. According to this the reaction is considered as an adsorption process, and in support of this assumption, several examples can be given where finely divided solids or colloid substances take up toxins or enzymes, in an irreversible manner (NERNST,[1] BILTZ,[2] LANDSTEINER[3]).

In reference to the formed antigens we must call attention to the following:

If certain cells, for example, bacteria, blood-corpuscles, and spermatozoa are injected into animals, then anti-bodies are formed which have been called *immune bodies* (also *amboceptors* or *sensibilizators*). By themselves the immune bodies are inactive, but form with *complements*, substances occurring in normal serum, so-called *cytotoxins*, which destroy the kind of cells active in their formation. These cytotoxins are called *bacteriolysins*, *hæmolysins*, etc., according to the kind of cells used. The immune bodies are specific in that they together with the complement only attack those cells from which they are formed and they are also stable against heat; the complements can act together with different immune bodies and are very unstable, as they are generally destroyed by heating to 56° C. for one-half hour. Other anti-bodies, produced under the influence of injected cells, show their action by flocking together and agglutinating the cells set free in their formation. These anti-bodies are called *agglutinins*.

In regard to the immune bodies, EHRLICH believes that they combine with those cells under whose influence they have been formed and also with the complements. They serve to fasten (amboceptors) the complement, which produces the real poisonous action, to the cells. The immune bodies correspond therefore to the haptophore groups of the toxins and the complements of the toxophores. According to BORDET the immune bodies act upon the cells in the way that the latter are sensitive toward the complements (sensibilizators).

If a certain immune serum is heated to 56° then, according to what has been given, the complement is destroyed and the serum now contains only the amboceptor of the original cyto-toxin and this amboceptor can be made active again by the addition of normal serum (complement).

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[1] Zeitschr. f. Elektrochem., **10**, 379 (1904).

[2] Ber. d. d. Chem. Gesellsch., **37**, 3147 (1904); Beitr. z. exp. Therapie, **1**, 30 (1905).

[3] Zeitschr. f. Chem. u. Ind. d. Koll., **2**, 221 (1907); Bioch. Zeitschr., **15**, 33 (1908).

If, therefore, an antigen of the corresponding immune serum be heated to 56° (amboceptor) and mixed with sufficient amount of normal serum (complement), then the complement is bound up so that when subsequently serum-free red-blood corpuscles and a certain quantity of immune serum, obtained by immunization with these and after losing its complement by heating to 56°, are added, no solution of the red-blood corpuscles (hæmolysis) takes place. If in the first mixture either the antigen or the corresponding amboceptors are absent then the complement is not combined and a hæmolysis occurs because the complement cannot unite with the hæmolytic amboceptors added. In this manner it has been attempted to determine the presence of an antigen or of amboceptors which fit with the antigen (method of complement deviation).

The protective substances formed by immunization can protect the organism against many fatal doses of the antigen and this protective power can be brought about by the parenteral introduction of the immune serum of another organism. The immunity is called *active* when the organism obtains the antigen and itself produces the corresponding protective substance. On the contrary the immunity is called *passive* if the organism receives the anti-body formed in another living being by active immunization.

During immunization under certain circumstances it is observed that a condition of super-sensitiveness toward the antigen exists. This super-sensitiveness occurs only toward the antigen used and is therefore specific. The same has been observed in using the soluble as well as the formed antigens. This mysterious phenomenon has been called *anaphylaxis*.

V. IONS AND SALT ACTION.

We have previously mentioned various processes which depend upon the influence of ions. To these belong the precipitation of suspension colloids by electrolytes as well as different catalytic processes. That in the last case we are dealing with the action of ions is proven by the fact that the velocity coefficient is proportional to the concentration of a certain kind of ion. Nevertheless it has been shown, that the velocity coefficient in the inversion of cane-sugar, by acid, is only proportional to the H ions when dilute acids are used. With greater concentration disturbances occur which can be ascribed to the action of the negative ions of the acids. The catalytic processes can be influenced by salts in a similar manner (salt action).

The enzyme action has shown itself proportional to the quantity of enzyme in certain cases. EULER [1] has attempted to show a correspondence between ion-

[1] Zeitschr. f. physik. Chem., **36**, 641 (1901).

action and enzyme action by the assumption that the enzymes cause an increase in those ions, which could cause the reaction without the presence of the enzyme. On the other hand J. LOEB [1] believes that the enzymes can also be electrolytically dissociated and that their action depends on the amount of ions. Thus pepsin is a weak base which forms a salt with the hydrochloric acid added and that this salt is more strongly dissociated than the base; for this reason the action of pepsin is increased by acid.

Many enzymotic processes are influenced by the presence of salts of the alkalies or alkaline earths. According to BIERRY, GIAJA and HENRI as well as PRETI [2] pancreatic juice dialyzed for a long time has no action upon starch, but becomes active again on adding NaCl or other salts. According to WOHLGEMUTH [3] the diastatic power of saliva is increased ten-fold by the addition of NaCl. The anions are the active part in both cases (see page 52 on co-enzymes). The strong retarding action which NaFl exerts upon the enzymotic cleavage of esters is also to be mentioned (LOEVENHART and PIERCE, AMBERG and LOEVENHART [4]).

Other actions of salts are also ascribed to ion-action. To these belong the experiments of DRESSER [5] according to which mercury salts, which are relatively strongly dissociated, have a poisonous action upon organic formations (yeast, frog heart), while potassium-mercury hyposulphite was nearly non-toxic. As the last-mentioned salt contains very few free Hg ions the posionous action of the first salt is ascribed to the ions. PAUL and KRÖNIG [6] have arrived at similar results by investigating the poisonous action of mercury salts upon spores. They found that K_2Cy_4Hg, which hardly contains any Hg ions, is much less poisonous than an equivalent solution of $HgCy_2$. The same conditions were observed by MAILLARD [7] for copper salts.

This leads us to the question as to the importance of water and the mineral bodies, which are of just as great moment for the life of the cells and their metabolism as the organic constituents. In regard to the water this follows from the fact that the animal body consists of about two-thirds water. If we also recall that water is of the greatest importance for the normal physical condition of the tissues, that the solution of numerous bodies and the dissociation of chemical compounds, that all flow of juices, all exchange of material, all supply of food, all growth or destruction and all removal of destructive prod-

[1] Bioch. Zeitschr., **19**, 534 (1909).

[2] Compt. rend. soc. biol., **60**, 479 (1906); **62**, 432, (1907); Bioch. Zeitschr., **4**, 1 (1907); **40**, 357 (1912).

[3] Bioch. Zeitschr., **9**, 1 (1908).

[4] Journ. of Biol. Chem., **2**, 397 (1907); **4**, 149 (1908).

[5] Arch. exp. Pathol. u. Pharm., **32**, 456 (1893).

[6] Zeitschr. f. physik. Chem., **31**, 411 (1896).

[7] Compt. rend. soc. biol., **50**, 1210 (1898).

ucts, are connected with the presence of water, and that besides this the water by its evaporation is an important regulator of temperature, it is evident that water must be a necessity of life.

The mineral substances found habitually in the cells of higher plants and of animals are *potassium, sodium, calcium, magnesium, iron, phosphoric acid, sulphuric acid, chlorine,* and perhaps also *iodine* (JUSTUS).[1] Besides, in certain cells or organs we also find *manganese, lithium, barium, silicium, fluorine, bromine,* and *arsenic.*

We are chiefly indebted to LIEBIG for showing that the mineral bodies are as important for the normal constitution of the organs and tissues, as well as for the normal performance of the processes of life, as the organic constituents of the body. The importance of the mineral constituents is evident from the fact that we know no animal tissue and no animal fluid which is free from mineral bodies, and also from the fact that certain tissues or tissue elements contain chiefly certain mineral bodies and not others. In regard to the alkali compounds this division is, in general, as follows: The sodium compounds occur chiefly in the fluids, while the potassium compounds occur especially in the form-elements. Corresponding to this, the cells contain chiefly potassium as phosphate, while they are less rich in sodium and chlorine compounds. The fundamental experiments of FORSTER[2] have shown us that inorganic salts, as constituents of the food are necessary for the animal organism.

We have already called attention to the importance for every organism of the salts for the production of a rather constant osmotic pressure. That the importance of the salts is not limited to the maintenance of the osmotic pressure follows from the fact that different salt solutions of the same osmotic pressure are not of the same value for the maintenance of the functional powers on extirpated organs. Since S. RINGER[3] showed that various organic structures retained their best functional activity in a solution which contained NaCl, $CaCl_2$ and KCl at the same time, various investigators have given the most suitable composition of such solutions. For the transfusion fluid for the mammalian heart LOCKE[4] suggests the following composition; NaCl

[1] Justus, Virchow's Arch., **170**, **176** and **190**. In regard to arsenic see the works of Gautier, Compt. rend., **129**, **130**, **131**, **139**; Bertrand. *ibid.*, **134**; Segale, Zeitschr. f. physiol. Chem., **42**; Kunkel, *ibid.*, **44**. In regard to the barium see Schultze and Thierfelder, Sitzungsber. d. Gesellsch. naturforsch. Freunde, 1905, No. 1, and in regard to lithium see Hermann, Pflüger's Arch., **109**; and in regard to manganese see Bradley, Journ. of Biol. Chem., **3**.

[2] Zeitschr. f. Biol., **9**, 297 (1873); **12**, 464 (1877).

[3] Journ. of Physiol., **6**, 154, 361 (1885); **7**, 118 (1886); **16**, 1, **17**, 23 (1895); **18**, 425 (1896).

[4] Centralbl. f. Physiol., **14**, 672 (1900).

0.9—1 per cent, $CaCl_2$ 0.02—0.024 per cent, KCl 0.02—0.042 per cent, $NaHCO_3$ 0.01.—0.03 per cent. Each of the salts NaCl, $CaCl_2$ and KCl individually has a poisonous action upon the organ but this action is counteracted by the presence of the two other salts (antagonistic salt action).

This neutralizing action of salts has been studied during recent years especially by J. LOEB and his collaborators. As general results it has been found that the most favorable quantity relations of the three salts NaCl, KCl and $CaCl_2$ for the maintenance of life is the same as exists in blood. Especially interesting are the experiments with the Fundulus heteroclitus, a genus of killifish. This fish, it is remarkable, can also live in distilled water and is therefore within wide limits, not dependent upon the osmotic pressure of the surrounding medium. For this reason it is specially suited for the study of the poisonous action of salts or mixture of salts. KCl in concentrations in which it exists in sea water acts as a poison upon these fishes, if it is alone in solution. The same is true for NaCl. On the contrary these fishes live for an indefinite time in a pure $CaCl_2$ solution in a concentration similar to sea water. One mol. KCl can be very nearly de-toxicated by 17 mol. NaCl or by $8\frac{1}{2}$ mol. Na_2SO_4. $\frac{1}{2}$ mol. K_2SO_4 is just as poisonous as 1 mol. KCl. The toxicity of the potassium salts is therefore dependent upon the K ions and the de-toxicating substance on the Na ion. $CaCl_2$ de-toxicates a KCl solution even when $\frac{1}{80}$ mol. $CaCl_2$ to 1 mol. KCl is present. $SrCl_2$ shows almost as great a de-toxicating action as $CaCl_2$. NaCl in concentrations, in which it occurs in sea-water can only be incompletely de-toxicated by KCl; only by the addition of $CaCl_2$ can the complete de-toxication be brought about. The poisonous action of acids upon Fundulus can be arrested by neutral salts.[1] Fundulus can accommodate themselves to a rise in temperature; a rise in temperature can be more easily endured when the concentration of the surrounding medium is raised at the same time (LOEB and WASTENEYS). Can fishes also accommodate themselves to an abnormal concentration of the surroundings as long as the rise in concentration takes place gradually? In both cases the accommodation, according to LOEB,[2] depends upon a slow proceeding process, possibly a tanning of the surface of the animal.

The fertilized eggs of the Fundulus develop, according to LOEB, just as well in water free from salt as in sea-water. If the fertilized eggs are placed in a NaCl solution of the same osmotic pressure as the sea-water

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[1] Bioch. Zeichr., 31, 450; 32, 155, 308; 33, 480. 489 (1911); 39, 167; 43, 181 (1912).

[2] Loeb and Wasteneys, Journ. of exp. Zool., 12, 543 (1913) and Loeb, Bioch. Zeitschr., 58, 391 (1913).

they die; the toxicity of the NaCl solution can be arrested by small quantities of almost any salt with polyvalent cations. Not only the salts of the alkaline earths, but also those of the heavy metals (for instance zinc sulphate or lead acetate) can neutralize the toxicity of the NaCl in proper concentration.[1] The eggs can develop in solutions which kill the completed fish.

The antagonistic action of salts upon organic structures depends, according to LOEB, upon the fact that the salts mixed in proper proportions causes a " tanning " of the protoplasmic surface of the cells whereby the cells become impermeable for certain destructive substances to which the salts also belong. The fertilized eggs of Fundulus can be tanned by NaCl+a heavy metal but not the completed fish.[2] Many observations indicate that the egg is more permeable after fertilization than before.[3]

Appendix: Determination of the Reaction of a Solution. The reaction of the solution, in which a chemical reaction takes place, plays an important rôle in many cases. As the acid or alkaline reaction of a solution depends upon the amount of H or OH ions it is often of importance to be able to determine the concentration of these ions in solution. These cannot be determined by titration with alkali or acid in the presence of organic salts. In this titration the existing equilibrium in the solution is disturbed and therefore also other decompositions occur besides the neutralization of H or OH ions. The quantity of alkali or acid used does not therefore correspond to the original concentration of H or OH ions.

According to the law of mass action there exists, between the H and OH ions formed by the dissociation of the water on the one hand and the concentration of the non-dissociated molecules on the other, the following equation

$$C_H . C_{OH} = K_1 . C_{H_2O}$$

where C_H, C_{OH} represents the concentration of the H and OH ions, C_{H_2O} the non-dissociated water molecules and K_1 a constant. As C_{H_2O} can only be considered as constant in certain dilute solutions we have $C_H . C_{OH} = K$, where K is called the dissociation constant of the water. As K is a constant it follows that the figures for C_H and C_{OH} can be calculated, if the other is known. As it is more convenient to determine C_H than C_{OH}, therefore C_H is also ordinarily determined for solutions

[1] Pflüger's Arch., 88, 68 (1901).

[2] Science, 34, 653 (1911).

[3] Lillie, Amer. Journ. of Physiol., 27, 289 (1911); McClendon, *ibid.*, 27, 240; Science, 32, 122, 317; Lyon and Shackell, *ibid.*, 32, 249 (1910).

with alkaline reaction. Complete investigations on this subject have been carried out by SÖRENSEN.[1] He found the value $10^{-14,14}$ for K at 18° C. C_H is determined in either of two ways. The best method, the electromotive, is based upon the electromotive force of gas chains, as developed by NERNST;[2] namely, if platinum foil covered with platinum black is introduced into a watery solution and this saturated with hydrogen, then a difference of electrical potential is produced between the platinum and the solution and this potential is theoretically proportional to the concentration of the hydrogen ions in the solution. We cannot give any further detail as to this theory or to the performance of the measurement of the difference in potential.[3] If the concentration of the hydrogen ions C_H is expressed in gram ions per liter by the figure 10^{-p}, then according to the suggestion of SÖRENSON the name hydrogen ion exponent and the symbol p_H is used for the numerical value of the exponents of this potence. The relationship between p_H and the electromotive force π at the contact between the platinum and the solution can be expressed graphically by a straight line; hence it follows that if π is known then p_H can be very easily found (the exponential line).

The other method used by SÖRENSEN[4] for the determination of C_H is a colorimetric method and depends on the use of indicators. After much investigation 20 indicators are recommended, of which certain ones require strictly fixed methods of use. As soon as more than a qualitative approximation is required then the shade of color produced by the indicator must be compared with a shade of color produced by the same indicator in a solution of known concentration of H ions. Such standard solutions which allow of a variation in the concentration of the H ions at one's pleasure have been given by SÖRENSEN, and the original article gives a table of curves from the corresponding value for p_H which can be read off, when the composition of a standard solution is known. The figure p_H for the standard solutions is determined by aid of the electromotive method. Standard solutions are selected so that they serve as natural protectors against too sudden changes in p_H (so called buffer)[5].

As above stated the dissociation constant according to SÖRENSEN for water is $10^{-14.14}$ at 18° C. or $C_H . C_{OH} = 10^{-14.14}$. In neutral reaction $C_H = C_{OH}$ and therefore $C_H = 10^{-7.07}$ or $p_H = 7.07$. Smaller values for p_H correspond to acid and greater values to alkaline reaction.

HASSELBACH[6] has suggested a modification of SÖRENSEN's method

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[1] Bioch. Zeitschr., 21, 131 (1909) also Ergebn. d. Physiol. Vol. 11.

[2] Zeitschr. f. physik. Chem., 4, 129 (1889).

[3] In regard to the determination see the work of Sörensen cited on page 74.

[4] Sörensen, Enzymstudien, Bioch. Zeitschr., 21, 253.

[5] *Ibid.*, 167.

[6] Bioch. Zeitschr., 30, 317 (1910).

for the electrometric determination of reaction in fluids containing carbon dioxide. By the aid of this method HASSELBACH and LUNDSGAARD [1] have made determinations of the reaction of the blood. From these it follows that at a temperature of 38.5° C. where the value for $p_H = 6.78$ corresponds to the neutral reaction the figure obtained for p_H with defibrinated ox-blood was 7.36 showing therefore a slight alkaline reaction. The influence of the respiratory variation in the CO_2 tension upon the H ion concentration of the blood is of a measurable size. The total blood has a greater H ion concentration than the serum at equal CO_2 tension but less than the blood corpuscles. For human blood saturated with CO_2 under 40 mm. tension at 38° C. LUNDSGAARD [2] found $p_H = 7.19$. MICHAELIS and DAVIDOFF [3] found the average values of normal venous blood for $p_H = 7.35$ at 37.5° C.

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[1] Biochem. Zeitschr., 38, 77 (1911).
[2] *Ibid.*, 41, 264 (1912).
[3] *Ibid.*, 46, 131 (1912).

CHAPTER II.

THE PROTEIN SUBSTANCES.

THE chief mass of the organic constituents of animal tissues consists of amorphous nitrogenized, very complex bodies of high molecular weight. These bodies, which are either proteins in a special sense or bodies nearly related thereto, take first rank among the organic constituents of the animal body on account of their great abundance. For this reason they are classed together in a special group which has received the name *protein group* (from πρωτεύω, I am the first, or take the first place). The bodies belonging to these several groups are called *protein substances*, although in a few cases the protein bodies in a special sense are designated by the same name.

The several *protein substances* [1] contain *carbon*, *hydrogen*, *nitrogen*, and *oxygen*. The majority contain also *sulphur*, a few *phosphorus*, and a few also *iron*. *Copper*, *chlorine*, *iodine*, and *bromine* have been found in some few cases. On heating the protein substances they gradually decompose, producing a strong odor of burned horn or wool. At the same time they produce inflammable gases, water, carbon dioxide, ammonia, and nitrogenized bases, besides many other substances, and leave a large quantity of carbon. On deep hydrolytic cleavage they yield abundance of α-monamino-acids of various kinds as decomposition products.

The nitrogen occurs in the protein bodies in various forms, and this is also revealed in the division of the nitrogen among the cleavage products. On boiling with dilute mineral acids we obtain (1) so-called amide nitrogen, which is readily split off as ammonia; (2) a guanidine residue which is combined with diaminovaleric acid as arginine, and which has also been called the urea-forming group; (3) basic nitrogen or diamino-acid nitrogen, or hexone bases nitrogen, which is precipitated by phosphotungstic acid as basic products (to which also the guanidine residue of arginine belongs); (4) monamino-acid nitrogen; and (5) the nitrogen

[1] See "Eiweisskörper," Ladenburg's Handwörterbuch der Chemie, **3**, 534–589, which gives a complete summary of the literature of protein substances up to 1885. The more recent literature may be found in O. Cohnheim, Chemie der Eiweisskörper, Braunschweig, 1911. See also Oppenheimer's Handbuch der Biochem. der Menschen und der Tiere, 1908.

in variable amounts which appears as humus-like melanoidins, which seem to be of only secondary formation as products of elaboration.

The quantitative division of the total nitrogen between the above five groups is different in the various protein substances, and moreover cannot be given with certainty, because of the above-mentioned melanoidin formation and the errors in the methods used.[1] The following gives at least an approximate idea of this division.[2] The loosely combined so-called amide nitrogen seems to be entirely absent in the protamines. In the gelatins we find 1–2 per cent, and 5–10 per cent in other animal protein substances,[3] in certain plant proteins, the prolamines (see page 106), 13–25 per cent of the total nitrogen is amide nitrogen. The guanidine nitrogen may amount in the protamines to 22–44 per cent of the total nitrogen, in the histones to 12–13 per cent, in the gelatins about 8 per cent, and in the other protein bodies about 2–5 per cent. As basic nitrogen precipitable by phosphotungstic acid (including the guanidine residue) we find 35–88 per cent in the protamines, 35–42.5 per cent in the histones, 15–30 per cent in the other animal protein substances. In the prolamines 3–6 per cent of the total nitrogen is found as products precipitable by phosphotungstic acid but in plant globulin (globulin of the wheat) indeed 37 per cent. The chief quantity of the nitrogen, 55–76 per cent, occurs, with the exception of the protamines, as the monamino-acid groups. The results for the melanoidin nitrogen vary so considerably that they will not be mentioned.

Recently D. v. SLYKE[4] has perfected a method which is based upon the deamidation of the amino-acids by HNO_2 (see below) and which allows of a still more detailed differentiation of the nitrogen partition. In this method the nitrogen of the ammonia, the melanines, the cystine, arginine, histidine, proline and oxyproline besides one-half of the tryphtophane nitrogen as well as the nitrogen of the remaining amino-acids can be specially determined.

From recent as well as older observations it follows as chief result that the nitrogen in the proteins occurs in such combinations so that on hydrolysis with acids, its chief amount splits off in the form of amino-acids.

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[1] See the work of Hausmann, Zeitschr. f. physiol. Chem., **27** and **29**; Henderson, *ibid.*, **27**; Kossel and Kutscher, *ibid.*, **30**; Kutscher, *ibid.*, **31**, **38**; Hart, *ibid.*, **33**; Gümbel, Hofmeister's Beiträge, **5**; Rothera, *ibid.*

[2] See the works given in footnote 1 and Blum, Zeitschr. f. physiol. Chem., **30**; Kossel, Ber. d. d. chem. Gesellsch., **34**, 3214; Hofmeister, Ergebnisse der Physiol., Jahrg. I, Abt. 1, 759, which also contains the literature; Osborne and Harris, Journ. Amer. Chem. Soc., **25**; and Gümbel, l.c.

[3] Skraup and v. Hardt-Stremayr, Monatsh. f. Chem., **29**, found lower results than other investigators and they found also that about two-thirds of the amide nitrogen was readily split off and one-third slowly.

[4] Ber. d. d. chem. Gesellsch, **43** and **44** and Journ. of biol. Chem., **9**, **10** and **12**.

By the action of nitrous acid upon proteins at least a partial deamidation takes place and so-called desamino proteins are obtained. The nitrogen expelled originated from the NH_2 groups according to the formula $RNH_2+HNO_2=ROH+N_2+H_2O$. The amount of such nitrogen is generally only small, 1–2 per cent, and for this reason it has been accepted that such groups only occur in small amounts in the proteins. This is probably true for a large number of proteins but not for all and as example of these we will recall that KOSSEL and CAMERON [1] have shown that those protamines which contain no other hexone base besides arginine although they have NH_2 groups at the ends in the guanidine residue $HN.CNH.NH_2$ of the numerous arginine groups, do not yield any nitrogen on using v. SLYKE's method while those protamines containing lysine do. We must be very careful in drawing certain conclusions from the results obtained by the action of nitrous acid upon proteins.

The nitrous acid can develop nitrogen from the NH_2 groups of the acid amides as well as from the NH_2 groups of the amino-acids. On the contrary no nitrogen is evolved in v. SLYKE's method from the guanidin groups and from the peptid combinations containing imid groups (see below). This is also the reason, as remarked above, why those protamines containing only arginine do not yield any nitrogen while those protamines which also contain lysine where there exist free NH_2 groups do give off nitrogen. On hydrolyzing these deamidized protamines and also other deamidized proteins we therefore do not obtain any lysin as shown by SKRAUP and collaborators and by LEVITES for certain proteins. The quantity of monamino-acid nitrogen is therefore in such cases found to be increased.

According to OSBORNE, LEAVENWORTH and BRAUTLECHT,[2] who worked with plant proteins, the splitting off of NH_3 on the acid hydrolysis of the proteins was very similar to the splitting off of NH_3 from the acid amide asparagine, so that the binding of NH_2 groups on the carboxyl groups seems very probable. The quantity of NH_3 split off in the hydrolysis ran parallel with the amount of asparagine and glutamic acid present and the quantity of NH_3, split off by hydrolysis with alkali corresponded nearly to the sum of the ammonia that was split off by acid hydrolysis and one-half of the arginine nitrogen. According to these investigators the NH_2 groups occur chiefly as acid-amide combinations.

A part of the nitrogen in the proteins occurs from the above, undoubtedly as NH_2 groups; the extent of this part, which is different in different proteins cannot be positively given. The chief mass of the nitrogen in the proteins, although other forms of binding occur, exists as imide-like combinations of amino-acids united together and this will be completely developed in the following pages.

The sulphur occurs in the different proteins in very different amounts. Certain of them, such as the protamines and apparently also certain

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[1] In regard to the action of nitrous acid upon proteins, their deamidation and cleavage products see C. Paal, Ber. d. d. Chem. Gesellsch., **29**; H. Schiff, *ibid.*, 1354; O. Loew, Chemiker Ztg., 1896 and O. Nasse, Pflüger's Arch., **6**; Treves and Salomone, Bioch. Zeitschr, **7**; Skraup, Monatsh. f. Chem., **27** and **28**, with Hoernes, *ibid.*, **27**, with Kaas, Annal. d. Chem. u. Pharm., **351**; Lampel, Monatsh. f. Chem., **28**; Traxl, *ibid.*, **29**; Levites, Zeitschr. f. physiol. Chem., **43**, and Bioch. Zeitschr., **20**; D. v. Slyke, foot-note 4, page 78; Kossel and Cameron, Zeitschr. f. physiol. Chem., **76**; Kossel and F. Weiss, *ibid.*, 78.

[2] Amer. Journ. of Physiol., **23**.

bacterial proteids,[1] are free from sulphur; some, such as gelatin and elastin, are very poor in sulphur, while others, especially horn substances, are relatively rich in sulphur. On hydrolytic cleavage with mineral acids, the sulphur of the protein substances is regularly, at least in part, split off as cystine (K. MÖRNER) or, with bodies poorer in sulphur, as cysteine (EMBDEN), but this, according to MÖRNER and PATTEN, is a secondary formation. From certain protein substances α-thiolactic acid (SUTER, FRIEDMANN, FRÄNKEL), which MÖRNER claims is also produced secondarily, mercaptans and sulphureted hydrogen (SIEBER and SCHOUBENKO, RUBNER), and a body having the odor of ethyl sulphide (DRECHSEL) have been obtained.[2]

A part of the sulphur separates as potassium or sodium sulphide on boiling with caustic potash or soda, and may be detected by lead acetate and quantitatively determined (FLEITMANN, DANILEWSKY, KRÜGER, FR. SCHULZ, OSBORNE, K. MÖRNER[3]). What remains can be detected only after fusing with potassium nitrate and sodium carbonate and testing for sulphates. The ratio between the sulphur split off by alkali and that not split off is different in various proteins. No conclusions can be drawn from this in regard to the number of forms of combination which the sulphur has in the protein molecule. As shown by K. MÖRNER, only about three-fourths of the sulphur in cystine can be split off by alkali, and the same is true for the cystine-yielding complex of the protein substances. If the quantity of lead-blackening sulphur in a protein body be multiplied by $\frac{4}{3}$, we obtain the quantity corresponding to the cystine sulphur in the body. By such calculation MÖRNER found in certain bodies, such as horn substance, seralbumin and serglobulin, that the quantity of cystine sulphur and total sulphur were identical, and therefore we have no reason for considering the sulphur in these bodies as existing in more than one form of combination. In other proteins, such as fibrinogen and ovalbumin, on the contrary, only one-half or one-third of the sulphur appeared as cystine sulphur.

Just as in the products of acid hydrolysis of proteins we know of two forms of oxygen bondage, the hydroxyl form OH and the carbonyl

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[1] See Nencki and Schaffer, Journ. f. prakt. Chem. (N. F.), **20**, and M. Nencki, Ber. d. d. chem. Gesellsch., **17**.

[2] K. Mörner, Zeitschr. f. physiol. Chem., **28**, **34**, and **42**; Patten, *ibid.*, **39**; Embden, *ibid.*, **32**; Suter, *ibid.*, **20**; Friedmann, Hofmeister's Beiträge, **3**; Sieber and Schoubenko, Archiv d. sciences biol. de St. Pétersbourg, **1**; Rubner, Arch. f. Hygiene, **19**; Drechsel, Centralbl. f. Physiol., **10**, 529; Fränkel, Sitzungsber. d. Wien. Akad., **112**, II *b*, 1903.

[3] Fleitmann, Annal. der Chem. und Pharm., **66**; Danilewsky, Zeitschr. f. physiol. Chem., **7**; Krüger's, Pflüger's Archiv, **43**; F. Schulz, Zeitschr. f. physiol. Chem., **25**; Osborne, Connecticut Agric. Expt. Station Report 1900; Mörner, l. c.

form in CONH; so according to TREAT B. JOHNSON [1] two analogous forms of sulphur bondage exist in the proteins, namely the mercaptan form SH as in cystine and the form NH.CH.CS.NH corresponding to the oxygen binding in the polypeptids (see page 86). He has in fact also prepared thio-polypeptides from glycocoll and these were analogous to the corresponding glycin polypeptids (see page 88) and like certain proteins gave H_2S on acid hydrolysis.

The constitution of the protein bodies is still unknown, although the great advances made in the last few years have brought us very much closer to the elucidation of the question. In studying the constitution of the protein bodies they have been broken up in various ways into simpler portions, and the methods used for this purpose have been of different kinds. In such decompositions, for which only purified proteins are to be used, first large atomic complexes—proteoses and peptones—are obtained which still have protein characteristics, and these then suffer further decomposition until finally we obtain simpler, generally crystalline, or at least well-characterized, end products.

As to the products obtained by hydrolytic *cleavage with mineral acids*, important investigations have been carried out by numerous older and more recent experimenters.[2] Besides certain acids, which will be mentioned later and which occur in few cases only, we obtain the following: monamino-acids such as glycocoll, alanine, aminovaleric acid, leucine, isoleucine, serine, aspartic and glutamic acids, cysteine and its disulphide cystine, phenylalanine, tyrosine, pyrollidine—and oxypyrollidine carboxylic acid, tryptophane and also the three hexone bases, histidine, arginine and lysine, the two latter being diamino-acids. Besides these also ammonia, sulphureted hydrogen, ethyl sulphide and melanoidins, which latter seem to be secondary products, have been obtained.

On the *hydrolysis with alkalies* we obtain, after a preliminary formation of intermediary steps which will be discussed later, chiefly the same cleavage products as in acid hydrolysis but with the exception that in the alkali hydrolysis a considerable part of the amino-acids become racemerized and therefore appear in optically inactive form while in the acid hydrolysis chiefly optically active acids are obtained. Because of the action of the alkali a part may suffer further decomposition which leads to the formation of simpler cleavage products and ammonia.

On fusing proteins with caustic alkali, ammonia, methyl mercaptan and other volatile products are evolved and other products are produced such as leucine,

[1] Journ. of biol. Chem., 9.

[2] In regard to the literature see O. Cohnheim, Chemie der Eiweisskörper, Braunschweig, 1911, and F. Hofmeister, Ergebnisse der Physiologie, Jahrg. I, Abt. 1, 759, 1902; E. Fischer, Untersuchungen über Aminosäuren, Polypeptide und Proteine (1899–1906), Berlin, 1906. See also special references.

from which then volatile fatty acids such as acetic acid, valeric acid and also butyric acid are formed, also tyrosine from which latter phenol is formed and indol and skatol.

Most proteins are split by *proteolytic enzymes* in the same manner as on hydrolysis with acids or alkalies, but more or less completely dependent upon the kind of enzymes. In the first place proteoses and peptones (see below) are formed, then also polypeptids and amino-acids of various kinds, in certain cases also oxyphenylethylamine, diamines, and a little ammonia and other bodies.

A great many substances are produced in the *putrefaction* of proteins. First the same bodies as are formed in the decomposition by means of proteolytic enzymes are produced, and then a further decomposition occurs with the formation besides ammonia, carbon dioxide and hydrogen, of a large number of bodies belonging in part to the aliphatic and in part to the aromatic and heterocyclic series.

To the aliphatic series belong volatile fatty acids and as shown by NEUBERG [1] and collaborators not only fatty acids of the normal chain but also with branched chains, also optically active acids, also succinic acid, methane, methyl mercaptan and others. To this series belongs also the two putrefaction bases cadaverine and putrescine, produced from the diamino acids, and also the so-called ptomaines or cadaver alkaloids which may originate, at least in part, from other tissue constituents and not from proteins.

The putrefactive products of the aromatic and heterocyclic series originate from the corresponding amino-acids. From tyrosine the aromatic oxy-acids such as *p*-oxyphenyl-propionic acid, the *p*-cresol, phenol and oxyphenylethylamine are formed. The phenylalanine is the mother substance of the phenylpropionic acid, the phenylacetic acid and the phenylethylamine. Indolpropionic acid, indolacetic acid, skatol and indol originate from the tryptophane (indolaminopropionic acid); the imidazolpropionic acid and imidazolethylamine originate from the histidine.[2]

By the moderate action of chlorine, bromine, or iodine upon proteins, these halogens enter into more or less firm combination with the proteins and according to the method of procedure we can prepare derivatives having different but constant amounts of halogens. The proteins are so changed that they do not split off sulphur on treatment with alkali, nor do they respond to MILLON'S or ADAMKIEWICZ-HOPKINS reaction. Side processes, oxidations and cleavages may also take place here. The most striking fact seems to be a substitution of hydrogen by iodine in the

[1] Bioch. Zeitschr., **37**, where the earlier works of Neuberg are cited.

[2] Ackermann, Zeitschr. f. physiol. Chem., **65**.

aromatic nucleus of tyrosine and also perhaps in the indol nucleus of tryptophane and the imidazol nucleus of histidine.[1] Halogen proteins occur, as will be shown later, in the animal kingdom, especially in the albuminoid group and indeed iodized tyrosine (3–5 di-iodotyrosine) has been isolated.

By the oxidation of protein by means of potassium permanganate, MALY obtained an acid, *oxyprotosulphonic acid*, C 51.21, H 6.89, N 14.59 S 1.77, O 23.24 per cent, which is not a cleavage product, but an oxidation product in which the group SH is changed into $SO_2.OH$. This acid does not give the proper color reaction with MILLON'S reagent, yields no tyrosine or indol, but gives benzene on fusing with alkali. On continued oxidation MALY obtained another acid, *peroxyproteic acid*, which gives the biuret reaction, but is not precipitated by most protein precipitants. The *oxyprotein* obtained by SCHULZ on the oxidation of protein by hydrogen peroxide is closely related to oxyprotosulphonic acid in composition and general characteristics, but contains lead-blackening sulphur and gives MILLON'S reaction. The oxyprotein is claimed to be a pure oxidation product, while in the production of oxyprotosulphonic acid SCHULZ claims that a cleavage takes place. According to BURACZEWSKI and KRAUZE the oxyprotosulphonic acid is a mixture of several substances. According to the investigations of v. FÜRTH [2] there exist at least three different peroxyproteic acids (from casein) which differ from each other by a different division of the nitrogen in the molecule. On treatment with baryta-water we find that they split off basic complexes and oxalic-acid groups, and new bodies, the *desamino-proteic acids*, which give the biuret reaction, are produced. These later acids, which on hydrolysis give benzoic acid but no diamino-acids, may be further oxidized, which is not true of the peroxyproteic acids, and yield a new group of acids, the *kyroproteic acids*, which give the biuret reaction, hold about one-half of their nitrogen (11.08 per cent total nitrogen) in acid-amide-like combination, but yield neither basic products nor benzoic acid.

On the oxidation of gelatin or protein with permanganate we also obtain oxaminic acid, oxamide, oxalic acid, oxaluric-acid amide, succinic acid, several volatile fatty acids, and guanidine, which was first shown by LOSSEN as an oxidation product.[3]

On the oxidation of gelatin by ferrous sulphate and hydrogen peroxide BLUMENTHAL and NEUBERG have obtained acetone as a product, and ORGLER the same from ovalbumin. The action of ozone upon casein has been studied

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[1] In regard to the action of halogens upon proteins see Loew, Journ. f. prakt. Chem. (N. F.), **31**; Blum, Münch. med. Wochenschr., 1896; Blum and Vaubel, Journ. f. prakt. Chem. (N. F.), **57**; Liebrecht, Ber. d. deutsch. chem. Gesellsch., **30**; Hopkins and Brook, Journ. of Physiol., **22**; Hopkins and Pinkus, Ber. d. deutsch. chem. Gesellsch., **31**; Hofmeister, Zeitschr. f. physiol. Chem., **24**; Kurajeff, *ibid.*, **26**; Oswald, Hofmeister's Beiträge, **3**; C. H. L. Schmidt, Zeitschr. f. physiol. Chem., **35, 36, 37**; Neuberg, Biochem. Zeitschr., **6**: Pauly and Gundermann, Ber. d. d. chem. Gesellsch., **41, 43**; Krzemecki, Chem. Centralbl., 1912; Pauly, Zeitschr. f. physiol. Chem., **76**.

[2] Maly, Sitzungsber, d. k. Akad. d. Wissensch., Wien, **91** and **97**. Also Monatshefte f. Chem., **6** and **9**. See also Bondzynski and Zoja, Zeitschr. f. physiol. Chem., **19**; Bernert, *ibid.*, **26**; Schulz, *ibid.*, **29**; Buraczewski and Krauze, *ibid*, **76**; v. Fürth, Hofmeister's Beiträge, **6**.

[3] Lossen, Annal. d. Chem. u. Pharm., **201**; Kutscher, Zeitschr. f. physiol. Chem., **32**; Zickgraf, *ibid.*, **41**; Seemann, *ibid.*, **44**; Kutscher and Schenck, Ber. d. d. chem. Gesellsch., **37** and **38**.

by HARRIES and LANGHELD [1] and the action of chlorine by HABERMANN and EHRENFELD and PANZER.[2]

Nitric acid gives various yellow products, which turn reddish-brown in alkaline solution. Of these we must especially mention the so-called xanthoprotein, besides nitrated proteoses and peptones. The xanthoprotein does not yield any tyrosine on acid hydrolysis and it does not give the Millon or the lead-blackening reactions. Among the cleavage products v. FÜRTH [3] has obtained a melanoidin substance, xanthomelanoidin.

On the nitration of the protamines (see below) KOSSEL [4] and co-workers have obtained nitroprotamines which give nitroarginine on hydrolysis which shows that the nitro groups have entered the guanidine groups of the arginine.

By the dry distillation of proteins we obtain a large number of decomposition products having a disagreeable burned odor, and a porous glistening mass of carbon containing nitrogen is left as a residue. The products of distillation are partly an alkaline liquid which contains ammonium carbonate and acetate, ammonium sulphide, ammonium cyanide, an inflammable oil, and other bodies, and a brown oil which contains hydrocarbons, nitrogenized bases belonging to the aniline and pyridine series, and a number of unknown substances.

The occurrence of protein substances which contain a carbohydrate group has been known for a long time. The nature of this carbohydrate, which can be split off by acid and which may amount to as much as 35 per cent, has been explained chiefly by the investigations of FRIEDRICH MÜLLER [5] and his students. They have shown that it is always an amino-sugar, and generally glucosamine and perhaps galactosamine as an exception. That so-called true proteins also yield a carbohydrate on hydrolytic cleavage was first shown by PAVY, using ovalbumin. The continued investigations of FR. MÜLLER, and others have demonstrated that in these cases the carbohydrate is also glucosamine. A carbohydrate complex, although sometimes only to a very slight amount, has been detected in other proteins, ovoglobulin, serglobulin, seralbumin, peaglobulin, albumin of the gramineæ, yolk-proteid, and fibrin. In other proteins, on the contrary, such, as edestin (of the hemp-seed) and casein, myosin, pure fibrinogen, and ovovitellin, carbohydrates have been sought for with negative results. All proteins hence do not contain a carbohydrate group, and future investigators must therefore decide whether the carbohydrate groups belong positively to the protein com-

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[1] Blumenthal and Neuberg, Deutsch. med. Wochenschr., 1901; Orgler, Hofmeister's Beiträge, 1; Harries and Langheld, Zeitschr. f. physiol. Chem., 51.

[2] Habermann and Ehrenfeld, Zeitschr. f. physiol. Chem., 32; Panzer, *ibid.*, 33 and 34.

[3] See Maly's Jahresber, 30, p. 24.

[4] Kossel and Kennaway, Zeitschr. f. physiol. Chem., 72, with E. Wechsler, *ibid.*, 78 and with F. Weiss, *ibid.*, 78.

[5] In regard to the literature on this subject see the work of Fr. Müller, Zeitschr. f. Biologie, 42, and Langstein, Ergebnisse der Physiologie, Jahrg. I, Abt. 1, 63, Zeitschr. f. physiol. Chem., 41, and Hofmeister's Beiträge, 6. See also Abderhalden, Bergell, and Dörpinghaus, Zeitschr. f. physiol. Chem., 41.

plex or whether they are united with the protein only as impurities. Several observations[1] show that in working with crystalline proteins a contamination with other protein substances is unfortunately not excluded, and this must not be lost sight of, especially as the quantity of carbohydrates obtained is often very small. In this connection we must call attention to the findings of OSBORNE and collaborators that on recrystallizing ovalbumin six times they found that the glucosamine content was reduced to 1.23 per cent while other investigators give 7–8–15 per cent. Under these circumstances we are not warranted in considering the carbohydrate groups as belonging to the carbon nucleus produced on the destruction of the real protein complex.

The previously mentioned methods used in studying the structure of the protein substances are not of the same value, but they in part substantiate each other. Of these we must mention the hydrolysis by means of boiling dilute mineral acids, or by proteolytic enzymes, as the best methods for obtaining the carbon nuclei in the protein molecule. The most important of the carbon nuclei obtained are as follows:

I. The Nuclei belonging to the Aliphatic Series.

A. *Sulphur free, but containing nitrogen:* 1. A *guanidine residue* (combined with ornithine as arginine). 2. *Monobasic monamino-acids:* Glycocoll, alanine, valine (amino valeric acid), leucine, and isoleucine. 3. *Bibasic monamino-acids:* Aspartic acid and glutamic acid. 4. *Oxymonamino-acids:* serine oxyaminosuccinic acid and oxyaminosuberic acid. 5. *Monobasic diamino-acids:* Diaminoacetic acid, ornithine (from arginine) and lysine. 6. *Oxydiamino-acids:* Oxydiaminosuberic acid, oxydiaminosebacic acid, diaminotrioxydodecanoic acid, caseanic and caseinic acids.

B. *Sulphurized:* Cysteine and its sulphide cystine, thiolactic acid (mercaptans, and ethyl sulphide).

II. The Nuclei belonging to the Carbocyclic Series.

Phenylalanine and tyrosine.

III. The Nuclei belonging to the Heterocyclic Series.

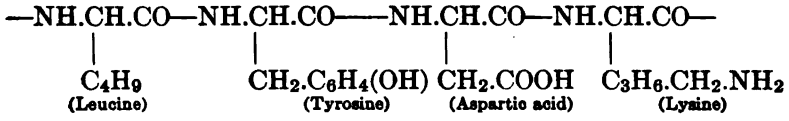
Proline, oxyproline, tryptophane and histidine.

In regard to these carbon nuclei it must be remarked that they are not all found in every protein body thus far investigated, and also that one and the same cleavage product, such, for example, as glycocoll, leucine, tryosine, etc., is obtained in very variable amounts from different protein substances.

It is very difficult to say to what extent all the above-mentioned carbon nuclei exist in the protein molecule. It is not inconceivable

[1] Osborne, D. B. Jones and Leavenworth, Amer. Journ. of Physiol., 24.

that in the hydrolysis certain carbon nuclei may be secondarily formed from others. Even if we admit the above, still it is undoubtedly true that the chief cleavage products of the protein substances are amino-acids. EMIL FISCHER has shown that the amino-acids have the property of readily grouping together when water is split off and the amide group of one amino-acid unites with the carboxyl group of the other. In accord with this behavior we can, as HOFMEISTER[1] and others have explained, but which was first proved by the epoch-making investigations of EMIL FISCHER, consider the proteins as chiefly formed by the condensation of amino-acids, where the amino-acids are united to each other by means of imino-groups according to the following scheme:



Such chaining of animo-acids is for the synthesis of protein-like bodies of the very greatest importance. The older statements of GRIMAUX, SCHÜTZENBERGER and PICKERING on the artificial preparation of protein-like substances where these investigators were able to prepare substances, which in many properties are similar to the proteins, from various amino-acids either alone or mixed with other bodies such as biuret, alloxan, xanthine, or ammonia. Of special interest are the investigations of CURTIUS and his collaborators, in which they were able to prepare synthetically the so-called *biuret base* (triglycyl-glycine ethyl ester) and subsequently many other bodies which were related to the proteins. The most important work on the chaining of amino-acids has been performed by E. FISCHER[2] and his pupils but especially by ABDERHALDEN. They have prepared a large number of complex bodies called *polypeptides* by FISCHER, which according to whether they contain two or more amino-acid groups united together, are called di-, tri-, tetrapeptides, etc. As examples of polypeptides we will mention—dipeptides: glycyl-tyrosine, alanylglycine, leucylglycine, leucylcystine, prolylphenylalanine,

[1] "Ueber den Bau des Eiweissmoleküls." Gesellsch. deutsch. Naturforscher und Aertze, Verhandl. 1902, and Ergebnisse der Physiologie, Jahrg. I, Abt. 1, 759.

[2] See Pickering, King's College, London, Physiol. Lab. Collect. Papers, 1897, which also cites Grimaux's work; also Journ. of Physiol., **18**, and Proceed. Roy. Soc., **60**, 1897; Schützenberger, Compt. rend., **106** and **112**; Curtius, Journ. f. prakt. Chem. (N. F.), **26** and **70**, and Ber. d. d. chem. Gesellsch., **37**; Fischer and collaborators, Untersuchungen über Aminosäuren, Polypeptide und Proteïne (1899–1906) Berlin 1906 and Ber. d. d. chem. Gesellsch., **39**, **40**, **41**, **42**, and Annal. d. Chem. u. Pharm., **354**, **357**, **363**, **365**, **369**, **375**; see also Abderhalden, Ber. d. d. chem., Gesellsch, **40** to **43** and Zeitschr. f. physiol. Chem., **72**, **75**, **77**.

leucylhistidine; tripeptides: di-glycylglycine, alanylglycyltyrosine, leucyltryptophylglutamic acid; tetrapeptides: glycylglutamyldiglycine, dileucylglycylglycine; pentapeptides: tetraglycylglycine and leucyltriglycylglycine; hexa- and heptapeptides: leucyltetraglycylglycine and leucylpentaglycylglycine. The most complex polypeptide thus far prepared is an octadecapeptide with 15 glycocoll and 3 leucine residues namely: *l*-leucyltriglycyl-*l*-leucyltriglycyl-*l*-leucyl-octaglycylglycine =

$$\mathrm{NH_2CH(C_4H_9)CO.[NHCH_2CO]_3.NHCH(C_4H_9)CO.}$$
$$\mathrm{[NHCH_2CO]_3.NHCH(C_4H_9)CO.[NHCH_2CO]_8.NHCH_2COOH.}$$

with the supposition that the amino-acids are here also combined together in the imide binding.

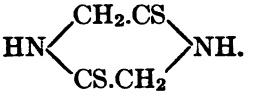
The large number of amino-acids isolated from the proteins make a large number of bindings possible. The number of possible combinations is still further increased by the fact that all the amino-acids with the exception of glycocoll contain at least one asymmetric carbon atom, and this leads to the possible formation of stereochemically different peptides. Thus in order to give a simple example, from two optically active amino-acids, four different isomeric forms of dipeptides may occur, namely (if we designate the optical antipodes by *d*- and *l*-) *dd*, *ll*, *dl* and *ld*. Of these forms two can form a racemic dipeptide, thus *d*-alanyl-*d*-leucine + *l*-alanyl-*l*-leucine and *d*-alanyl-*l*-leucine + *l*-alanyl-*d*-leucine. As the proteins are optically active and on hydrolysis yield chiefly optically active amino-acids, those polypeptides which can be built up from the natural amino-acids of the proteins are of special importance in the study of the constitution of the proteins.

Most of the artificial polypeptides are constructed from monamino-mono-carboxylic acids, but polypeptides have also been prepared which contain diamino-acids or amino-dicarboxylic acids, and in this way the number of possible polypeptides becomes still greater. With an aminodicarboxylic acid such as aspartic acid, other amino-acids can be bound with one carboxyl group or with both, but also, if we start with asparagine, they can be anchored with the amide group. If we start from the acid amides we can also obtain a peptide which still contains the $CONH_2$ group and on total hydrolysis yields NH_3, like most proteins. A polypeptide of this kind is the tripeptide, glycl-*l*-asparaginyl-*l*-leucine prepared by E. Fischer and Koenigs.

$$\begin{array}{c}\mathrm{NH_2CH_2CO.NHCHCO.NHCH(C_4H_9)COOH}\\ |\\ \mathrm{CH_2CONH_2}\end{array}$$

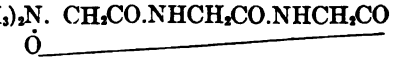
In consideration of the form of binding of the sulphur in the proteins it is interesting to consider the preparation of thiopolypeptids as performed

by TREAT B. JOHNSON.[1] He has prepared the following: thioglycylglycinthioamide $NH_2CH_2CS.NHCH_2CSNH_2$ which is analogous to glycylglycinamide, $NH_2CH_2CO.NHCH_2CONH_2$ and also dithiopiperazine



Polypeptides of higher amino-fatty acids such as α-aminolaurylalanine, α-aminolaurylleucine and others have been prepared by HOPWOOD and WEIZMANN.[2] These peptids are different from the so-called *lipopeptids* prepared by BONDI and his collaborators[3] which are not chains of only amino-acids but combinations between a high fatty acid, such as lauric- or palmitic acid and an amino-acid (glycocoll or alanine) or a dipeptide (lauryl-alanylglycine).

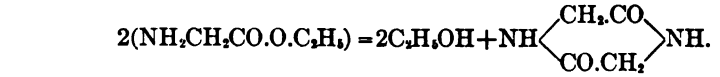
Methylated polypeptides such as methyl- and dimethylleucylglycine (E. FISCHER and GLUUD) and betaindiglycylglycine



(ABDERHALDEN and KAUTZSCH) are also known.[4] Amides of amino-acids and dipeptides have been prepared by BERGELL[5] and his co-workers.

The methods used by E. FISCHER in the synthetical preparation of polypeptides are chiefly as follows:

The first dipeptide prepared by him, glycylglycine, he obtained from glycocoll ethyl ester which in water is transformed into a diketopiperazine, glycine anhydride, according to the following equation:



By the action of dilute alkali upon this anhydride with the taking up of water the glycylglycine $NH_2CH_2CO.NHCH_2COOH$ is formed, and according to this principle other dipeptides can also be prepared.

Another method which has much greater application consists in the anchoring of an amino-acid to a halogen of an acid radical, for example, by the action of brompropionyl bromide or chloride upon glycocoll according to the following equation:

$$CH_3CHBrCOCl+NH_2CH_2COOH=HCl+CH_3CHBrCO.NHCH_2COOH$$

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[1] Journ. of biol. Chem., **9**.

[2] See Chem. Centralbl., 1911.

[3] Bioch. Zeitschr., **17** and **23**; see also Abderhalden and C. Funk, Zeitschr. f. physiol. Chem., **65**.

[4] Fischer and Gluud, Annal. d. Chem. u. Pharm., **369**; Abderhalden and Kautzsch, Zeitschr. f. physiol. Chem., **72** and **75**.

[5] *Ibid.*, **64**, **65** and **67**.

(brompropionyl glycine). On subsequent treatment with ammonia the halogen (Br) is replaced by NH_2 and the dipeptide alanylglycine

$$CH_3CHNH_2CO.NHCH_2COOH+NH_4Br$$

is obtained. . By the second action of brompropionylchloride and then treatment with NH_3 we introduce a new alanyl group and the tripeptide alanyl-alanyl glycine is prepared. By the action of a halogen derivative of an acid radical another amino-acid residue can be introduced, and the chain of amino groups can be thus extended.

The prolongation of the chain on the other side, namely, at the carboxyl, FISCHER has accomplished by chlorination of the amino-acids by special treatment with phosphorus pentachloride. The carboxyl is thus transformed into COCl, while the acid at the same time fixes a molecule of HCl, for example CH_3CHNH_2HCl (COCl)

Just as in the case of the carboxyl group of an amino-acid, so also can a polypeptide or its halogen acyl combination be chlorinated and then combined with a new amino-acid, or a new peptide. As an example, FISCHER, from α-bromisocapronyldiglycyl glycine, first prepared α-bromisocapronyldiglycylglycyl chloride, and then with diglycylglycine he obtained the heptapeptide leucylpentaglycylglycine,

$$C_4H_9CH(NH_2)CO.(NHCH_2CO)_5.NHCH_2COOH.$$

For the various combinations of the optically active amino-acids to polypeptides it was important to possess methods of preparation of these amino-acids, and for this purpose FISCHER in many cases used the so-called WALDEN'S reversion. This consists in that one optically active amino-acid, for example the *l*-form, is transformed into the corresponding halogen fatty acid by the action of nitrosyl bromide, yielding the optical antipode the *d*-form. By the action of ammonia the *d*-amino-acid is now obtained which in the above-mentioned manner can be retransformed into the *l*-form. Thus from *d*-leucine we first obtain *l*-bromisocaproic acid and then by the action of ammonia *l*-leucine and in the preparation of the polypeptides the same occurs. Thus, for example, if by reversion *d*-leucine is changed first into *l*-bromisocapronyl chloride, if this last is combined with *l*-leucine, then we obtain the dipeptide *l*-leucyl-*l*-leucine. On combination with diglycylglycine the tetrapeptide *l*-leucyl-diglycyl glycine is produced. WALDEN'S reversion does not take place with all amino-acids; other methods can also be used to obtain the optical antipodes, such as the preparation of the alkaloidal salts of the benzoyl or formyl combinations of the racemic amino-acids.

The β-naphthalinsulpho combination of the polypeptides and peptones may serve, as FISCHER, ABDERHALDEN and FUNK [1] have shown, in explaining the structure of these bodies. By the action of β-naphthaline sulphochloride the NH_2 groups existing at the beginning of the chain in the amino-acids react therewith and on subsequent total hydrolysis this naphthaline-sulpho combination remains unsplit. Thus for instance we can differentiate between glycylalanine and alanylglycine because after hydrolysis in the first case we obtain naphthalinsulphoglycine and alanine and in the second naphthalin-sulphoalanine and glycocoll (glycine). Tyrosine may, depending upon whether the NH_2 as well as the OH groups are free or not or if only one is available, yield di- or mononaphthalinsulpho-derivatives and in this way we can also draw conclusions as to the structure of tyrosine containing peptides.

The previously mentioned deamidation method of van Slyke (page 78) where oxyacids are formed by the action of HNO_2 upon the NH_2 groups can also give

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[1] E. Fischer and Abderhalden, Ber. d. d. Chem. Gesellsch., **40**; Abderhalden and C. Funk, Zeitschr. f. physiol. Chem., **64**.

certain conclusions as to the structure of the peptides by comparing the hydrolytic products before and after deamidation.

A comparison of the artificially prepared polypeptides with the proteins, and especially with the cleavage products of these last, the so-called preoteoses and peptones, is of great interest in several respects, especially in connection with certain reactions. For instance there are several polypeptides which give the biuret reaction which is characteristic of the proteins in general, and also several (polypeptides containing tyrosine), which give MILLON'S reaction (see further on). The above-mentioned octadecapeptide is precipitated by phosphotungstic acid, tannin and ammonium sulphate; we also know tri- and pentapeptides containing tyrosine, which are very similar in properties to the proteoses.

The behavior of the polypeptides with proteolytic enzymes is of great interest. As this interesting question will be thoroughly treated in other chapters (I and VIII) it is sufficient here to recall that the possibility that polypeptides as well as proteins are hydrolyzed by the same enzymes, yielding amino-acids, is a weighty proof of the probability that in the proteins the amino-acid chains are of the same kind as in the polypeptides.

A very important support for such a view is found in the occurrence of polypeptides among the cleavage products of proteins, a find which to a certain extent forms the reverse of the above-mentioned syntheses. Such polypeptides are chiefly di- but also tri- and tetrapeptides. They have been obtained in the hydrolytic products of silk waste, silk fibroin and elastin (FISCHER, ABDERHALDEN), gelatin (LEVENE, WALLACE and BEATTY) and of gliadin (OSBORNE and CLAPP)[1]. Of special interest in this connection are those polypeptides which like glycyl-*d*-alanine, *d*-alanyl-glycine, glycyl-*l*-tyrosine, *l*-prolyl-*l*-phenylalanine and *d*-alanyl-glycyl-*l*-tyrosine, are identical with the corresponding synthetically prepared polypeptides or at least very closely related.

We have therefore conclusive reasons for the assumption that in the proteins, peptide bindings chiefly occur, i.e., a combination of the α-amino-acids by means of the imide binding. It is also possible that other linking may occur, and FISCHER has also given expression to such a possibility. Besides the above-mentioned imide binding another kind must also without doubt exist in the proteins, namely, the anchoring of the urea-forming group (the guanidine residue) with the ornithin (diamino-valeric acid) by the imide binding. This imide linking is

[1] Fischer and Abderhalden, Sitz. Ber. d. d. Berl. Akad. d. Wissensch, **30**, and Ber. d. d. Chem. Gesellsch., **39**, **40**; Abderhalden, Zeitschr. f. physiol. Chem., **62**, **63** and **72**; Levene and Wallace, *ibid.*, **47**, with Beatty, Ber. d. d. Chem. Gesellsch., **39**, and Bioch. Zeitschr., **4**; Osborne and Clapp, Amer. Journ. of Physiol., **18**.

not, like the α-amino-acids, broken by trypsin, but rather by an enzyme arginase, discovered by KOSSEL and DAKIN.[1]

If the proteins are considered as consisting chiefly of peptide-like complexes consisting of amino-acids united and containing also several NH_2 groups at the ends, it is readily understood that the proteins are amphoteric electrolytes, like the amino-acids, which form salts with bases as well as with acids and undergo hydrolytic dissociation. As we also accept the theory that the protein molecule contains a large number of COOH as well as NH_2 groups, it follows that the proteins may be polybasic acids as well as polyacidic bases. The different proteins act in this regard somewhat differently, thus the protamines are strongly basic while casein behaves strikingly acid, and others take a certain mean position. It is unfortunately impossible to base a classification of the proteins upon this behavior, as well as upon chemical constitution. The general properties, such as solubility and precipitation properties, are too uncertain to aid us, and especially as in the investigations of proteins we, as a rule, cannot decide whether we are dealing with a pure or with a contaminated substance, namely, with mixtures. Experience has shown that the solubility and precipitation properties of the proteins are strongly influenced by the presence of other bodies, and under such circumstances a proper classification, as demanded by science, is impossible. On the other hand, a classification is important, and as the ones used up to the present time were based upon the solubility and precipitation properties, we give the following schematic summary of the chief groups of protein bodies:

I. Simple Proteins.

A. True Albuminous Bodies or Proteids.

Albumins	*Seralbumin*, *Lactalbumin*, and others.
Globulins	*Fibrinogen*, *Serglobulins*, and others.
Phosphoproteins (Nucleoalbumins)	*Ovovitellin*, *Casein*, and others.
(Coagulated proteins.)	
Histones.	
(Protamines?)	

[1] Zeitschr. f. physiol. Chem., **41**.

B. ALBUMINOIDS OR ALBUMOIDS.

Keratins.
Elastin.
Collagen and glutin.
Reticulin.

(Fibroin, Sericin, Collin, Cornein, Spongin, Byssus, and others.)

C. CLEAVAGE PRODUCTS OF TRUE ALBUMINOUS BODIES.

Alkali and Acid Albuminates.
Proteoses, Peptones, Polypeptides.
(Amino-acids.)

II. Compound Proteins.

Glycoproteins..... { *Mucin substances,* / *Ichthulin,* and others.

Nucleoproteins.

Chromoproteins..... { *Hæmoglobin,* / *Hæmocyanin.*

As there are two classifications recognized by English-speaking scientists we will give the classifications adopted by the American Physiological Society and the American Society of Biological Chemists and also the British Medical Association.

Classification adopted by the American Physiological Society and the American Society of Biological Chemists:

I. Simple Proteins.

A. **Albumins.**
B. **Globulins.**
C. **Glutelins.**
D. **Prolamins** (*Alcohol-soluble proteins*).
E. **Albuminoids.**
F. **Histones.**
G. **Protamines.**

II. Conjugated Proteins.

A. **Nucleoproteins.**
B. **Glycoproteins.**
C. **Phosphoproteins.**
D. **Hæmoglobins.**
E. **Lecithoproteins.**

III. Derived Proteins.

1. Primary Protein Derivatives.

A. **Proteans.**
B. **Metaproteins.**
C. **Coagulated proteins.**

2. Secondary Protein Derivatives.

A. **Proteoses.**
B. **Peptones.**
C. **Peptides.**

Classification of proteins adopted by the British Medical Association:

I. Simple Proteins.

1. **Protamines.**
2. **Histones.**
3. **Albumins.**
4. **Globulins.**
5. **Glutelins.**
6. **Alcohol-soluble proteins.**
7. **Scleroproteins.**
8. **Phosphoproteins.**

II. Conjugated Proteins.

1. **Glucoproteins.**
2. **Nucleoproteins.**
3. **Chromoproteins.**

III. Products of Protein Hydrolysis.

1. **Infraproteins.**
2. **Proteoses.**
3. **Peptones.**
4. **Polypeptides.**

To this summary must be added that we often find in the investigations of animal fluids and tissues protein substances which do not fall in with the above schemes, or are classified only with difficulty. At the same time it must be remarked that bodies will be found which seem to rank between the different groups, hence it is very difficult to sharply divide these groups.

I. Simple Proteins.

A. True Albuminous Bodies.

The albuminous bodies are never-failing constituents of the animal and vegetable organisms. They are especially found in the animal body, where they form the solid constituents of the muscles and of the blood-serum, and they are so generally distributed that there are only a few animal secretions and excretions, such as the tears, the perspiration, and perhaps the urine, in which they are entirely absent or occur only in traces.

All albuminous bodies contain *carbon, hydrogen, nitrogen, oxygen* and *sulphur*;[1] a few contain also *phosphorus*. *Iron* is generally found in traces in their ash. The elementary composition of the different albuminous bodies varies a little, but the variations are within relatively close limits. For the better-studied animal albuminous bodies the following composition of the ash-free substance has been found:

C	50.5 —54.6	per cent
H	6.5 — 7.3	"
N	15.0 —17.6	"
S	0.5 — 2.2	"
P	0.42— 0.85	"
O	21.50—23.50	"

The animal proteids are odorless, tasteless, and ordinarily amorphous. The crystalloid spherules (*Dotterplättchen*) occurring in the eggs of certain fishes and amphibians, do not consist of pure proteids, but of albuminous bodies containing large amounts of lecithin, which seem to be combined with mineral substances. Crystalline proteids[2] have been prepared from the seeds of various plants, and crystallized animal proteids (see seralbumin and ovalbumin, Chapters V and XII) can be readily prepared. In the dry condition the proteids appear as white powders, or when in thin layers as yellowish, hard, transparent plates. A few are soluble in water, others only soluble in salt or faintly alkaline or acid solutions, while others are insoluble in these solvents. Solutions of proteids are optically active and turn the plane of polarized light to the left. All proteids when burned leave an ash, and it is therefore ques-

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[1] See foot-note 1, p. 80.

[2] See Maschke, Journ. f. prakt. Chem., **74**; Drechsel, *ibid.* (N. F.), **19**; Grübler, *ibid.* (N. F.), **23**; Ritthausen, *ibid.* (N. F.), **25**; Schmiedeberg, Zeitschr. f. physiol. Chem., **1**; Weyl., **1**; *ibid.*, **1**.

tionable whether there exists any proteid body which is soluble in water without the aid of mineral substances. Nevertheless it has not been thus far successfully proved that a native proteid body can be prepared perfectly free from mineral substances without changing its constitution or its properties.[1]

As previously stated, the albuminous bodies are amphoteric electrolytes, and are polyacidic bases as well as polybasic acids. The base- and acid-combining powers of various proteids have been the subject of numerous investigations which cannot be given in short. In regard to various methods used in such investigations as well as to the dissociation of protein salts we refer especially to the work of T. B. ROBERTSON.[2]

The proteids can be salted out from their neutral solutions by neutral salts (NaCl, Na_2SO_4, $MgSO_4$, $[NH_4]_2SO_4$, and many others) in sufficient concentrations. By this salting out the properties remain unchanged and the process is reversible, as on diminishing the concentration of the salt the precipitate redissolves. The various proteids act in an entirely different manner toward the same salt, and also for one and the same proteid the behavior toward different neutral salts is different, as some cause a precipitate, while others on the contrary do not precipitate.

The behavior of various proteids with one and the same salt, such as $MgSO_4$ or $(NH_4)_2SO_4$, is often made use of in the isolation of the proteid, and special methods of separation are based upon fractional precipitation. It has been shown that these methods may lead to great errors, and give good results only under special conditions.[3]

The conditions are different from those of salting out, when the proteid solution is precipitated by salts of the heavy metals. Here the precipitates (often called metallic albuminates) are not true combinations in constant proportions, but are rather to be considered as loose adsorption compounds of the proteid with the salt.[4] These reactions are irreversible in so far that dilution with water or removal of the salt by means of dialysis does not restore the unchanged proteid. On the other hand the precipitate, at least in certain cases may be redissolved in an excess of the salt solution or of the proteid solution, and in this sense the process is a reversible one.

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[1] See E. Harnack, Ber. d. d. chem. Gesellsch., **22**, **23**, **25**, and **31**; Werigo, Pflüger's Archiv, **48**; Bülow, *ibid.*, **58**; Schulz, Die Grösse des Eiweissmoleküls, Jena, 1903.

[2] Ergeb. d. Physiol. 10; Journ. of physical Chem., **14**, **15**, and Journ. of biol. Chem., **9**.

[3] See Cohnheim, Chemie der Eiweisskörper, 3. Aufl., 1911; Pinkus, Journ. of Physiol., **27**; Pauli, Hofmeister's Beiträge, **3**, p. 225; Halsam, Journ. of Physiol., **32**.

[4] See Galeotti, Zeitschr. f. physiol. Chem., **40**, **42**, **44**, and **48** and Bonamartini and Lombardi, *ibid.*, **58**. See also the opposed views of Lippich, *ibid*, **74**.

The precipitation of proteids and also other soluble proteins by salts stands in close relation to their colloidal nature, and in this connection we refer to what has been said in Chapter I. The proteids do not as a rule diffuse through animal membranes, or only to a very slight extent, and hence have in most cases a pronounced colloidal nature in GRAHAM'S sense. They belong to the hydrophile colloids; their solutions show properties in common with those of typical colloids and also true solutions. Certain of them, especially the peptones and a few proteoses, which will be discussed later, seem to occupy an intermediate position, as their solutions are characterized by a lesser viscosity and greater diffusibility and filtration ability, are not readily precipitable by alcohol or coagulable by heat, and are only partially precipitable by salts.

The solutions (or suspensions) of proteids in water, the proteid hydrosols, are converted by various means into proteid hydrogels. Of these means we must specially mention the following: flocking out with salts, precipitation with alcohol, gelatinization of a gelatin solution on cooling, and coagulation by the action of enzymes or heat.

Those proteids which occur, according to the common views, preformed in the animal fluids and tissues, and which have been isolated from these by indifferent chemical means without losing their original properties, are called *native proteids*. New modifications having other properties can be obtained from the native proteids by heating, by the action of various chemical reagents such as acids, alkalies, alcohol, and others, as well as by proteolytic enzymes. These new proteids are called *modified* (" *denaturierte* ") *proteids*, to differentiate them from the native proteids.

The precipitation with alcohol is a reversible reaction, as the precipitate redissolves on subsequent dilution with water. The proteids are changed by the action of alcohol, some readily and quickly, others with difficulty and very slowly; the proteid then becomes insoluble in water and is modified.

On heating a solution of a native proteid it is modified at a different temperature for each different proteid. With proper reaction and other favorable conditions, for instance in the presence of neutral salts, most proteids can in this way be precipitated in a solid form as coagulated proteid. The hydrosol is converted into hydrogel, but as a modification takes place, this process is irreversible. The temperature at which coagulation occurs is a variable one for the same protein under different conditions of the experiment. The various temperatures at which coagulation of different proteids occurs in neutral solutions containing salt have in many cases given us good means for detecting and separating proteids. The views in regard to the use of these means are somewhat

divided [1] and the same applies to the question as to manner of heat coagulation and the conditions under which it takes place.

The heat coagulation of a protein solution is dependent upon the hydrogen ion concentration of the solution. According to MICHAELIS and co-workers the optimum of the hydrogen ion concentration falls in the coagulation of a protein solution (precipitation of the modified protein) with the isoelectric point of the solution and the optimum of the flocking is not changed in regard to the hydrogen ion concentration by changes in the protein concentration. According to SÖRENSEN and JÜRGENSEN,[2] on the contrary the optimal hydrogen ion concentration is the same as contained in a solution of the pure protein in pure water caused by the electrolytic dissociation of this protein and is therefore independent of the protein concentration. This hydrogen ion concentration, according to these workers diminishes during the heat coagulation which they consider as a proof of the diminution in the protein concentration of the solution.

A modification can be brought about also by the action of acids, alkalies, or salts of the heavy metals, in certain cases by water alone, and also by the action of alcohol, chloroform (SALKOWSKI), and ether, by violent shaking (RAMSDEN[3]), etc.

An adsorption of proteids by a suspension colloid such as silicic acid, colloidal ferric hydroxide and kaolin, can easily take place, and indeed the proteid of a solution can be removed by the use of colloidal ferric hydroxide or shaking with kaolin (RONA and MICHAELIS [4]). That the proteids can serve as preventives in the precipitation of suspension colloids has been mentioned in Chapter I. In the same manner a mastic suspension is protected from the precipitating action of an electrolyte by an excess of a proteid solution, while the reverse may be brought about, namely, a proteid solution can be precipitated by a large quantity of mastic emulsion in the presence of a proportionately small amount of electrolyte. The method for the removal of proteid from solutions, as suggested by MICHAELIS and RONA,[5] is based upon this behavior.

We have already discussed in Chapter I the electric charge of the proteins under various conditions and the migration of these in electric fields of currents.

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[1] See Halliburton, Journ. of Physiol., 5 and 11; Corin and Berard, Bull. de l'Acad-roy. de Belg., 15; Haycraft and Duggan, Brit. Med. Journ., 1890, and Proc. Roy. Soc. Edin., 1889; Corin and Ansiaux, Bull. de l'Acad. roy. de Belg., 21; L. Frédericq, Centralbl. f. Physiol., 3; Haycraft, *ibid.*, 4; Hewlett, Journ. of Physiol., 13; Duclaux, Annal. Institut Pasteur, 7. In regard to the relationship of the neutral salts to the heat coagulation of albumins see also Starke, Sitzungsber. d. Gesellsch. f. Morph. u. Physiol. in München, 1897; Pauli, Pflüger's Arch., 78.

[2] See Ergeb. d. Physiol., 12 which contains the pertinent literature.

[3] See Salkowski, Zeitschr. f. physiol. Chem., 31; Fr. Krüger, Zeitschr. f. Biologie, 41; Loew and Aso, Bull. Coll. Agric., Tokio, 4; Ramsden, Zeitschr. f. physik. Chem., 47 and Arch. f. (anat. u.) Physiol., 1894.

[4] Biochem. Zeitschr., 5.

[5] Biochem. Zeitschr., 2, 3 and 4.

The determination of the molecular weight of the proteids has been attempted by various methods which are more or less uncertain.[1] There is no doubt that the molecular weight of the proteids is very high, but the statements about the size vary considerably. For the true proteids thus far investigated, values ranging from 4000—6000—10,000 have been found.

The general reactions for the proteids are very numerous, but only the most important will be given here. To facilitate the study of these, they have been divided into the two following groups. It must be remarked that the precipitation reactions are not only applicable for the soluble true proteids but also, more or less, for other soluble proteins in general. The color reactions are applicable to all soluble or insoluble proteins with few exceptions, which will be mentioned later.

Precipitation Reactions of the Proteid Bodies.

1. *Coagulation Test.* An alkaline proteid solution does not coagulate on boiling, and a neutral solution only partly and incompletely; the reaction must therefore be acid for coagulation. The neutral liquid is first boiled and then the proper amount of acid added carefully. A flocculent precipitate is formed, and with proper technique the filtrate should be water-clear. If dilute acetic acid be used for this test, the liquid must first be boiled and then 1, 2, or 3 drops of acid added to each 10–15 cc., depending on the amount of proteid present, and boiled before the addition of each drop. If dilute nitric acid (25 per cent) be used, then to 10–15 cc. of the previously boiled liquid 15–20 drops of the acid must be added. If too little nitric acid be added, a soluble combination of the acid and proteid is formed, which is precipitated by more acid. A proteid solution containing a small amount of salts must first be treated with about 1 per cent NaCl, since the heating test may fail, especially on using acetic acid, in the presence of only a slight amount of proteid.

2. *Precipitation by Alcohol.* The solution must not be alkaline, but must be either neutral or faintly acid. It must, at the same time, contain sufficient quantity of neutral salts.

3. *Neutral Salts,* such as Na_2SO_4 or NaCl, when added to saturation precipitate certain proteids but not others. *Ammonium sulphate* when dissolved to saturation in the liquid is considered as the general precipitant for proteids. In the presence of free acetic or hydrochloric acid the above-mentioned salts, NaCl or Na_2SO_4, in sufficient concentration, are also general precipitants for the proteids.

4. *Precipitation by Metallic Salts* such as copper sulphate, ferric chloride, neutral and basic lead acetate (in small amounts), mercuric

[1] See especially F. N. Schulz, Die Grösse des Eiweissmolecüle, Jena, 1903.

chloride and others. On this is based the use of proteids as antidotes in poisoning with metallic salts.

5. *Precipitation by Mineral Acids at Ordinary Temperatures.* The proteids are precipitated by the three ordinary mineral acids in proper amounts, but not by orthophosphoric acid. If nitric acid be placed in a test-tube and the proteid solution be allowed to flow gently thereon, a white opaque ring of precipitated proteid will form where the two liquids meet (HELLER'S albumin test).

6. *Precipitation by the so-called Alkaloid Reagents.* To these belong the precipitation by *metaphosphoric acid* and by *hydroferrocyanic acid,* which is carried out by the aid of potassium ferrocyanide in a liquid containing acetic acid; precipitation by *phosphotungstic acid* or *phosphomolybdic acid* in the presence of free mineral acids; precipitation by *potassium-mercuric iodide* or *potassium-bismuth iodide* in solutions acidified with hydrochloric acid; precipitation by *tannic acid* in acetic acid solutions. The absence of neutral salts or the presence of free mineral acids may prevent the appearance of the precipitate, but after the addition of a sufficient quantity of sodium acetate the precipitate will in both cases appear; precipitation by *picric acid* in solutions acidified by organic acids. Proteids are also precipitated by *trichloracetic acid* in 2-5 per cent solutions, by *phenol, salicyl sulphonic acid, nucleic acid, taurocholic acid* and by *chondroitin sulphuric acid* in acid solutions.

Color Reactions for Proteid Bodies.

1. *Millon's Reaction.*[1] A solution of mercury in nitric acid containing some nitrous acid gives a precipitate with proteid solutions which at the ordinary temperature is slowly, but at the boiling-point more quickly, colored red; and the solution may also be colored a feeble or bright red. Solid albuminous bodies, when treated by this reagent, give the same coloration. This reaction is due to the tyrosine and is also given by other monohydroxyl benzene derivatives. According to O. NASSE[2] it is best to use a solution of mercuric acetate which is treated with a few drops of a 1 per cent solution of potassium or sodium nitrite; previous to use a few drops of acetic acid are added.

2. *Xanthoproteic Reaction.* With strong nitric acid the albuminous bodies give, on heating to boiling, yellow flakes or a yellow solution.

[1] The reagent is prepared in the following way: 1 pt. mercury is dissolved in 2 pts. nitric acid (of sp.gr. 1.42), first cold and then warmed. After complete solution of the mercury add 1 volume of the solution to 2 volumes of water. Allow this to stand a few hours and decant the supernatant liquid.

[2] See O. Nasse, Sitzungsb. d. Naturforsch. Gesellsch. zu Halle, 1879, and Pflüger's Arch., 83; see also Vaubel and Blum, Journ. f. prakt. Chem. (N. F.), 57.

After making alkaline with ammonia or alkalies the color becomes orange-yellow, due to the nitroderivatives of the benzene and indol groups.

3. *Adamkiewicz's Reaction.* If a little proteid is added to a mixture of 1 vol. concentrated sulphuric acid and 2 vols. glacial acetic acid a reddish-violet color is obtained slowly at ordinary temperatures, but more quickly on heating. According to HOPKINS and COLE[1] this reaction takes place only on using glacial acetic acid containing glyoxylic acid. According to them it is better to use a solution of glyoxylic acid, which can be readily prepared by adding sodium amalgam to a concentrated solution of oxalic acid and filtering after the discharge of the gas. A dilute aqueous solution of the acid or some of the solid acid is added to the proteid solution and sulphuric acid allowed to flow down the side of the test-tube, when the reddish-violet color will appear at the point of contact of the two liquids or on shaking the mixture. This color reaction, which is generally called the ADAMKIEWICZ-HOPKINS reaction depends upon the tryptophane and therefore gelatin (which does not contain any tryptophane) does not give this reaction.

As further color reactions we will mention: 4. *Biuret Test.* If a proteid solution be first treated with caustic potash or soda and if then a dilute copper-sulphate solution be added drop by drop, first a reddish then a reddish-violet, and lastly a violet-blue, color is obtained. 5. Proteids are soluble on heating with *concentrated hydrochloric acid,* producing a violet color, and when they are previously boiled with alcohol and then washed with ether (LIEBERMANN[2]) they give a beautiful blue solution. This blue color is due, according to COLE,[3] to a contamination of the ether with glyoxylic acid, which reacts with the tryptophane groups split off by the hydrochloric acid. The violet color obtained with proteins not purified with ether is also considered as a tryptophane reaction with the furfurol (oxymethylfurfurol) formed from the hexose containing protein by the action of the concentrated hydrochloric acid. Reaction 6 with *concentrated sulphuric acid* and *sugar* (in small quantities) is explained in the same way. The beautiful red coloration is connected with the formation of furfurol from the sugar. 7. With *p*-dimethylaminobenzaldehyde and concentrated sulphuric acid the proteids give a beautiful reddish-violet or deep-violet coloration (O. NEUBAUER and E. ROHDE[4]). Other aldehydes also give color reactions by virtue of the tryptophane group in proteins. Other reactions are 8; ARNOLD'S *reaction*[5] is a purple-violet coloration which the proteins give

[1] Proceed. Roy. Soc., **68**.
[2] Centralbl. f. d. med. Wissensch., 1887.
[3] Journ. of Physiol., **30**.
[4] Zeitsche. f. physiol. Chem., **44**.
[5] Arnold, Zeitschr. f. physiol. Chem., **70**.

with sodium nitroprusside and ammonia. This reaction is not given by all proteins and is due to the cystine groups. 9, ABDERHALDEN and SCHMIDT'S reaction with triketohydrindenhydrate which gives a blue coloration on boiling. The triketohydrindenhydrate (also called "Ninhydrin") reacts with all compounds which have an amino group in the α-position to the carboxyl, is according to ABDERHALDEN and SCHMIDT [1] an excellent reagent for the detection of dialyzable amino-acids and non-biuret giving amino-acid derivatives. They have been able to detect by this reagent such non-biuret giving substances in the dialysate on the dialysis of different animal fluids. They have also determined the delicacy of this reagent with different amino-acids.

The biuret reaction is not only given by protein substances, but also by many other bodies. According to H. SCHIFF [2] this reaction occurs with those bodies containing amino groups, $CONH_2$, $CSNH_2$, $C(NH)NH_2$ or also CH_2NH_2, united either directly by their carbon atoms or by means of a third carbon or nitrogen atom. As examples of such bodies we can mention several diamines or amino-amides, such as oximide, biuret, glycinamide, α- and β-aminobutyramide, aspartic-acid amide, etc., although we are not certain as to the conditions necessary for the bringing about of this reaction. The biuret reaction alone is therefore no proof as to the protein nature of a substance—for example, urobilin gives a very similar color reaction—and a protein substance can still retain its protein nature, as by the action of nitrous acid or by a splitting off of ammonia, although it does not give the biuret reaction.

The delicacy of the various reagents differs for the different proteids, and on this account it is impossible to give the degree of delicacy for each reaction for all proteids. Of the precipitation reactions, HELLER'S test (if we eliminate the peptones and certain proteoses) is recommended in the first place for its delicacy, though it is not the most delicate reaction, and because it can be performed so easily. Among the precipitation reactions, that with basic lead acetate (when carefully and exactly executed) and with alcohol and the reactions given under 6, are the most delicate. The color reactions 1 to 4 show great delicacy in the order in which they are given.[3]

No proteid reaction is in itself characteristic, and, therefore, in testing for proteids one reaction is not sufficient, but a number of precipitation and color reactions must be employed.

For the quantitative estimation of coagulable proteids the determination by boiling with acetic acid can be performed with advantage, for by operating carefully, it gives exact results. Treat the proteid solution with a 1–2 per cent common-salt solution, or if the solution contains large amounts of proteid dilute with the proper quantity of the above salt solution, and then carefully neutralize with acetic acid. Now deter-

[1] Zeitschr. f. physiol. Chem., **72** and **85**.

[2] Ber. d. d. chem. Gesellsch., **29** and **30**.

[3] In regard to the precipitation and color reactions of proteids with aniline dyes see Heidenhain, Pflüger's Arch., **90**, **96**.

mine the quantity of acetic acid necessary to completely precipitate the proteids in small measured portions of the neutralized liquid which have previously been heated on the water-bath, so that the filtrate does not respond to HELLER'T test. Now warm a larger weighed or measured quantity of the liquid on the water-bath, and add gradually the required quantity of acetic acid, with constant stirring, and continue heating for some time. Filter, wash with water, extract with alcohol and then with ether, dry, weigh, incinerate, and weigh again. With proper work the filtrate should not give HELLER'S test. This method serves in most cases, and especially so in cases where other bodies are to be quantitatively estimated in the filtrate.

In many cases good results may be obtained by precipitating all the proteid with tannic acid and determining the nitrogen in the washed precipitate by means of KJELDAHL'S method. On multiplying the quantity of nitrogen found by 6.25 we obtain the quantity of proteid. Many other methods for the quantitative estimation of proteins have been suggested.

The removal of proteids from a solution may in most cases be performed by boiling with acetic acid. Small amounts of proteid which remain in the filtrates may be separated by boiling with freshly precipitated lead carbonate or with ferric acetate, as described by HOFMEISTER.[1] If the liquid cannot be boiled, the proteid may be precipitated by the very careful addition of lead acetate, or by the addition of alcohol. If the liquid contains substances which are precipitated by alcohol, such as glycogen, then the proteid may be removed by trichloracetic acid as suggested by OBERMAYER and FRÄNKEL.[2] Recently MICHAELIS and RONA have suggested a method for the removal of proteids by using kaolin, colloidal ferric hydrate or a mastic emulsion. The principle of these methods has already been given on page 97 and in regard to the practical execution of the method we refer to the works there cited.

In the precipitation of proteid as well as the quantitative estimation by means of heat, it must be borne in mind, as shown by SPIRO,[3] that several nitrogenous substances, such as piperidine, pyridine, urea, etc., disturb the coagulation of the proteids.

Synopsis of the Most Important Properties of the Different Groups of Albuminous Bodies.

As it is not possible to base the classification of the different proteid groups according to their constitution, we are obliged to make use of their different solubilities and precipitation properties in their general characterization. As there exist no sharp differences between the various groups in this regard it is impossible to draw a sharp line between them.

Albumins. These bodies are soluble in water in neutral reaction and are not precipitated by the addition of a little acid or alkali. They are

[1] Zeitschr. f. physiol. Chem., **2** and **4**.

[2] Obermayer, Wien. med. Jahrb., 1888; Fränkel, Pflüger's Arch., **52** and **55**.

[3] Zeitschr. f. physiol. Chem., **30**.

precipitated by the addition of large quantities of mineral acids or metallic salts. Their solution in water coagulates on boiling in the presence of neutral salts, but a weak saline solution does not. If NaCl or $MgSO_4$ is added to saturation to a neutral solution in water at the normal temperature or at 30° C. no precipitate is formed; but if acetic acid is added to this saturated solution the albumins readily separate. When ammonium sulphate is added to one-half saturation the albumin solutions are not precipitated at ordinary temperatures. Of all the native proteids the albumins are the richest in sulphur, containing from 1.6 per cent to 2.2 per cent. So far as they have been investigated they do not yield any glycocoll on acid hydrolysis.

Globulins. These substances are, as a rule, insoluble in water, but dissolve in dilute neutral salt solutions. The globulins are precipitated unchanged from these solutions by sufficient dilution with water, and on heating they coagulate. The globulins dissolve in water on the addition of very little acid or alkali, and on neutralizing the solvent they precipitate again. The solution in a minimum amount of alkali is precipitated by carbon dioxide, but the precipitate may in certain cases be redissolved by an excess of the precipitant. The neutral solutions of the globulins containing salts are partly or completely precipitated on saturation with NaCl or $MgSO_4$ in substance at normal temperatures, depending upon the kind of globulin. The globulins are completely precipitated by half-saturating with ammonium sulphate. The globulins contain an average amount of sulphur generally not below 1 per cent. As a difference between the albumins and globulins the latter yield glycocoll among the hydrolytic cleavage products, and according to OBERMAYER and WILLHEIM [1] they contain fewer NH_2 groups at the end of the chain, as determined by formol titration, as compared to the total number of N-atoms.

A sharp line cannot be drawn between the albumins and globulins from their properties and this is shown from the researches of MOLL,[2] which show that by the action of dilute alkalies and warmth upon seralbumin it attains the properties of serglobulin. It is evident that we are here dealing with a change of the external properties of the albumins to a greater similarity to those of the globulins, and not with a true transformation of the albumin, which is free from glycocoll, into globulin which contains glycocoll. The same follows from the observations of others.[3] This is an instructive example of the subordinate importance the solubility and precipitation properties have in the differentiation of various groups of proteids.

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[1] Bioch. Zeitschr., **38**.

[2] Moll, Hofmeister's Beiträge, 4 and 7; also Breinl, Arch. f. exp. Path. u. Pharm., **65**.

[3] Obermayer and Willheim, l. c.; R. Gibson, Journ. of biol. Chem., **12**.

It is just as difficult to draw a sharp line between the globulins and albuminates as it is between the globulins and albumins. Several globulins are very readily changed by the action of very little acid, as also by standing under water when in a precipitated condition, into albuminates, and then become insoluble in neutral salt solutions. OSBORNE,[1] who has closely studied this property in connection with edestin (from hemp-seed), considers the globulin, "globan," which has been made insoluble in salt solution, as an intermediate step in the formation of the albuminate which is produced by the hydrolytic action of the H ions of water or of the acid.

Phosphoproteins are a group of phosphorized proteids which occur extensively in the animal and plant kingdoms and which include the *nucleoalbumins* and the little-studied *lecithalbumins*.

Nucleoalbumins. These proteids behave like rather strong acids, are nearly insoluble in water, but dissolve easily with the aid of a little alkali and, in the entire absence of phosphatides, contain also phosphorus. Certain of the nucleoalbumins, resemble the globulins by their solubility and precipitation properties. Others resemble the albuminates, but differ from both of these groups by containing phosphorus. They stand close to the nucleoproteins by their content of phosphorus, but differ from these in not yielding any purine bases on cleavage. It has not yet been found possible to obtain from the neucleoalbumins any proteid-free pseudonucleic acids corresponding to the nucleic acids, but only acids rich in phosphorus, which always give the proteid reactions.[2] For this reason the nucleoalbumins cannot be classed as compound proteins. In peptic digestion a proteid rich in phosphorus can be split off from most nucleoalbumins, and this has been called *para-* or *pseudonuclein*. The claim made that the pseudonuclein is a combination of proteid with metaphosphoric acid has been shown to be incorrect by the investigations of GIERTZ.[3]

The separation of pseudonuclein in peptic digestion is no doubt characteristic of the nucleoalbumin group, but the non-appearance of the pseudonuclein precipitate does not entirely exclude the presence of a nucleoalbumin. The extent of such a formation is dependent upon the intensity of the pepsin digestion, the degree of acidity, and the relation between the nucleoalbumins and the digestive fluids. The separation of a pseudonuclein may, as shown by SALKOWSKI, not occur even in the digestion of ordinary casein, and WRÓBLEWSKI and others[4] did not obtain any pseudonuclein at all in the digestion of the casein from human milk. The most essential characteristic of this group of proteids is that they contain phosphorus, and that the purine bases are absent in their cleavage products.

[1] Zeitschr. f. physiol. Chem., **33**.

[2] Levene and Alsberg, *ibid.*, **31**; Salkowski, *ibid.*, **32**; Levene, *ibid.*, **32**; A. Reh, Hofmeister's Beiträge, **11**; Dietrich, Bioch. Zeitschr., **22**.

[3] Giertz, Zeitschr. f. physiol. Chem., **28**.

[4] Salkowski, Pflüger's Arch., **63**; Wróblewski, Beiträge zur Kenntnis des Frauenkaseïns, Inaug.-Diss., Bern, 1894.

The nucleoalbumins are often confounded with nucleoproteins and also with phosphorized glucoproteins. From the first class, they differ by not yielding any purine bases when boiled with acids, and from the second group by not yielding any reducing substance on the same treatment. The best studied member of this group is the casein of milk, which will be discussed in detail in chapter XIII.

NEUBERG and POLLAK [1] have artifically prepared phosphoproteins by the action of phosphorus oxychloride upon an alkaline solution of lactalbumin or blood globulin. The product obtained from lactalbumin was rather close to casein in regard to composition and other properties.

Lecithalbumins. In the preparation of certain protein substances, products are often obtained containing lecithin, and this lecithin (see the phosphatides, Chapter IV) can be removed only with difficulty or incompletely by a mixture of alcohol and ether. Ovovitellin (Chapter XII) is such a protein body containing considerable lecithin, and HOPPE-SEYLER considers it a combination of proteid and lecithin. Similar substances occur in fish-eggs. These last lecithalbumins often have the solubilities of the globulins and are readily soluble in dilute salt solutions. The behavior of the nucleoalbumin of the eggs of the perch shows how easily this solubility may be changed. This nucleoalbumin, which contains considerable amounts of lecithin, is readily soluble in dilute NaCl solution, but at ordinary temperatures it is changed by 0.1 per cent HCl almost instantaneously and without splitting off lecithin, so that it becomes insoluble in dilute salt solutions (HAMMARSTEN). LIEBERMANN [2] has obtained proteids containing lecithin as an insoluble residue on the peptic digestion of the mucous membrane of the stomach, liver, kidneys, lungs, and spleen. He considers them as combinations of proteid and lecithin and calls them *lecithalbumins*. Further investigation of these bodies is desirable.

MAYER and TERROINE [3] have shown that from lecithin emulsified in water and a dialyzed solution of ovalbumin or dialyzed blood serum a precipitate can be obtained which has some similarity to the lecithalbumins, but which in other respects is so strikingly different that we are not justified in calling this precipitate lecithalbumin.

Nothing characteristic has thus far been found which differentiates this group from others in the quantity of amino-acids split off on hydrolysis. The members of this group differ essentially among themselves, e.g., vitellin yields glycocoll while casein does not.

In order to give a review of the three above-mentioned groups of proteids we give (page 106) a tabulation of the amounts of the amino-acids

[1] Ber. d. d. chem. Gesellsch., **43** and Bioch. Zeitschr., **26**.

[2] Hoppe-Seyler, Med. chem. Untersuch., 1868; also Zeitschr. f. physiol. Chem., **18**, 479; Hammarsten, Skand. Arch. f. Physiol., 17; Liebermann, Pflüger's Archiv, **50** and **54**.

[3] Compt. rend. soc. biol., **62**.

obtained on cleavage, but we must bear in mind that the figures, because of the difficulty in the quantitative estimation, are not quite exact, but must be considered as minimum values. As a representative of the globulin group we give fibrin, which is a coagulated globulin; and as representative of the phosphoprotein group, besides casein also ovovitellin, although not quite pure. The results are based on 100 parts of the substances.

The proteins occurring in the plant kingdom either in the seeds or tuberes belong chiefly to the *globulins*, which correspond essentially in properties to the animal globulins. Besides these many other less abundant proteins occur, which like the *albumins* are soluble in water, while toward certain salts they behave like globulins. It is not clear whether the phosphorized plant proteids contain their phosphorus as impurities or whether they are the same as the animal phosphoproteins. In seeds there occur also proteins, which are not represented in the animal kingdom and of these we must especially mention the *prolamines*. They are soluble in alcohol and besides this they are characterized by not yielding any lysine on hydrolysis.

	Lact-albumin.[1]	Ser-albumin.[2]	Ov-albumin.	Ser-globulins.[2]	Fibrin.[5]	Casein.[7]	Vitellin.[8,9]
Glycocoll	0.0	0.0	0.0	3.5	3.0	0.00	1.1[8]
Alanine	2.5	2.7	2.2	2.2	3.6	1.50	0.75[9]
Valine	0.9	—	2.5	—	1.0	7.20	2.40[8]
Leucine	19.4	20.0	10.7	18.7	15.0	9.35	11.0[8]
Isoleucine	—	—	—	—	—	1.43[10]	—
Serine	—	0.6	—	—	0.8	0.50	—
Aspartic acid	1.0	3.1	2.2	2.5	2.0	1.39	2.13[9]
Glutamic acid	10.1	7.7	9.1	8.5	10.4	15.55	12.95[9]
Cystine	—	2.5[3]	0.3[3]	1.51[3]	1.17[3]	0.07	—
Phenylalanine	2.4	3.1	5.17	3.8	2.5	3.20	2.8[8]
Tyrosine	0.85	2.1	1.77	2.5	3.5	4.50	3.37[9]
Proline	4.0	1.04	3.56	2.8	3.6	6.70	4.18[9]
Oxyproline	—	—	—	—	—	0.23	—
Tryptophane	3.07[11]	—	—	—	—	1.50	—
Histidine	—	—	1.71	—	—	2.50	1.90[9]
Arginine	—	—	4.91	—	3.0[6]	3.81	7.46[9]
Lysine	—	—	3.76	—	4.0[6]	5.95	4.81[9]
Ammonia	—	—	1.34	—	—	1.60	1.25[9]

[1] Abderhalden and H. Pribram, Zeitschr. f. physiol. Chem., **21.**
[2] Abderhalden, Lehrb. d. physiol. Chem., 1909.
[3] K. Mörner, Zeitschr. f. physiol. Chem., **34.**
[4] Osborne and co-workers, Amer. Journ. of Physiol, **24.**
[5] Abderhalden and Voitinovici, Zeitschr. f. physiol. Chem., **52.**
[6] Kutscher, Endprodukte der Trypsin Verdauung, Habit. Schrift., Marburg, 1899.
[7] Osborne and Guest, Journ. of biol. Chem., **9.**
[8] Abderhalden and Hunter, Zeitschr. f. physiol. Chem., **48.**
[9] Osborne and D. B. Jones, Amer. Journ. of Physiol., **24.**
[10] Levene and D. v. Slyke, Journ. of biol. Chem., **6.**
[11] Colorimetric determinations by Fasal, Bioch., Zeitschr, **44.**

In the tabulation of the hydrolytic products of plant proteins we give *edestin*, of the hemp-seed, and *legumin* of the pea as examples of globulins. The other three, *hordein* of barley, *gliadin* of wheat and *zein* from corn belong to the prolamine group.

	Edestin.[1]	Legumin.[4]	Hordein.[6]	Gliadin.[8]	Zein.[10]
Glycocoll	3.8	0.38	0.0	0.68[9]	0.0
Alanine	3.6	2.08	0.43	2.0	9.79
Valine	5.6[2]	1.0[5]	0.13	3.34	1.88
Leucine	20.9	8.0	5.67	6.62	19.55
Serine	0.33	0.53	—	0.13	1.02
Aspartic acid	4.5	5.3	—	0.58	1.71
Glutamic acid	18.74[3]	13.8	43.19[7]	43.66	26.17
Cystine	0.25	—	—	0.45	—
Phenylalanine	2.4	3.75	5.03	2.35	6.55
Tyrosine	2.1	1.55	1.67	1.20	3.55–10.1[11]
Proline	1.7	3.22	13.73	13.22	9.04
Oxyproline	2.0	—	—	—	—
Tryptophane	0.38[12]	—	—	1.00	—
Histidine	1.1	2.42	1.28	0.61	0.82
Arginine	11.7	10.12	2.16	3.16	1.55
Lysine	1.0	4.29	0.00	0.00	0.00
Ammonia	—	1.49	4.87	5.22	3.61

Coagulated Proteins. Proteins may be converted into the coagulated condition by different means: by heating, by the action of alcohol, especially in the presence of neutral salts, by chloroform, ether, and metallic salts, and by the prolonged shaking of their solutions and in certain cases, as in the conversion of fibrinogen into fibrin (Chapter V), by the action of an enzyme. The nature of the processes which take place during coagulation is unknown. The coagulated albuminous bodies are insoluble in water, in neutral salt solutions, and dilute acids or alkalies, at normal temperature. They are dissolved and converted into albuminates by the action of dilute acids or alkalies, especially on heating.

Coagulated proteins also seem to occur in animal tissues. We find, at least in many organs such as the liver and other glands, proteins

[1] Abderhalden, Zeitschr. f. physiol. Chem., **37** and **40**.
[2] Levene and D. v. Slyke, Journ. of biol. Chem., **6**.
[3] Osborne and Biddle Amer. Journ. of Physiol, **26**.
[4] Osborne and Clapp, Journ. of biol. Chem. **3**.
[5] Abderhalden and Babkin, *ibid.*, **47**.
[6] Osborne and Clapp, Amer. Journ. of Physiol., **19**.
[7] Osborne and Jones, *ibid.*, **26**.
[8] Osborne and Guest, Journ. of biol. Chem., **9**.
[9] Abderhalden and Samuely, Zeitschr. f. physiol. Chem., **44**.
[10] Osborne, Jones and Clapp, Amer. Journ. of Physiol., **26**.
[11] Kutscher, Zeitschr. f. physiol. Chem., **38**.
[12] Fasal, Bioch. Zeitschr., **44**.

which are not soluble in water, dilute salt solutions, or very dilute alkalies, and only dissolve after being modified by strong alkalies.

Histones are basic proteins which stand to a certain extent between the strongly basic protamines (see below) and the true proteins. Their content of nitrogen varies between 16.5 and 19.8 per cent, and in certain instances is not higher than in other proteins, especially vegetable proteins. According to KOSSEL and KUTSCHER and LAWROW they are, on the contrary, richer in basic nitrogen, and especially yield more arginine than other proteins. KOSSEL first isolated a peculiar protein substance from the red corpuscles of goose blood which was precipitated by ammonia, and because of its similarity in certain regards to the peptones (in the old sense) he called it histone. At the present time a number of very different bodies are described as histones, such as those obtained from nucleohistone (LILIENFELD), from hæmoglobin (*globin* according to SCHULZ), from mackerel spermatozoa (*scombron* according to BANG), from the codfish (*gadushistone* according to KOSSEL and KUTSCHER), from the burbot (*lotahistone*, EHRSTRÖM), and from the sea-urchin (*arbacin*, MATHEWS), although probably not all are true histones, especially the above mentioned globin.[1]

Sulphur has been found in those histones in which it has been tested for, but they do not, at least not all, give the lead-blackening test with alkali and lead acetate. They give the biuret test, but as a rule only a faint MILLON'S reaction. The goose-blood histone first studied by KOSSEL gives the three following reactions: First, the neutral salt-free solution does not coagulate on boiling; second, gives a precipitate with ammonia which is insoluble in an excess of the precipitant; third, gives a precipitate with nitric acid which disappears on heating and reappears on cooling.

The different histones behave differently with these three reactions, and hence they are not specific. On the other hand, all histones seem to be precipitated from neutral solution by alkaloid reagents, and they also produce precipitates in protein solutions. These two reactions are likewise not specific for the histones, as the protamines have a similar behavior. The histones differ from the protamines by having a much lower content of basic nitrogen, and also probably by always containing sulphur. True proteins, as OSBORNE'S[2] edestan, also give these two reactions; therefore it is impossible by qualitative tests alone to identify

[1] Kossel, Zeitschr. f. physiol. Chem., 8, and Sitzungbers. der Gesellsch. zur Beförd. d. ges Naturwiss. zu Marburg, 1897; Kossel and Kutscher, *ibid.*, 1900, and Zeitschr. f. physiol. Chem., **31**; Lawrow, *ibid.*, **28**, and Ber. d. d. chem. Gesellsch., **34**; Lilienfeld, Zeitschr. f. physiol. Chem., 18; Schulz, *ibid.*, **24**; Bang, *ibid.*, **27**; Ehrström, *ibid.*, **32**; Mathews, *ibid*, **28**.

[2] Zeitschr. f. physiol. Chem., **33**.

a substance as a histone with positiveness. The large content of basic nitrogen and of arginine is not a sure point of difference between histones and other bodies. Histone yields little more than 40 per cent basic nitrogen, while a heteroproteose yields about the same, namely, 39 per cent. Histone yields 14–15.5 per cent arginine (gadushistone), and the lotahistone only 12 per cent. The vegetable proteid excelsin is just as rich in arginine, namely, 14.14 per cent (OSBORNE and CLAPP[1]). The characteristics of the histones, according to KOSSEL, are the above-given reactions and the high amount of hexone bases, especially arginine. The arginine nitrogen amounts to about 25 per cent of the total nitrogen, the lysine N = 7–8.5 per cent and the histidine N = 1.8–4.5 per cent. No proteids, with the exception of certain protamines, are known for the present, which contain as much arginine and lysine as the histones. On hydrolytic cleavage the histones, like other proteins, but unlike the protamines, yield a large number of monamino-acids. ABDERHALDEN and RONA[2] obtained from thymus histone the following: leucine 11.8, alanine 3.46, glycocoll 0.50, proline 1.46, phenylalanine 2.20, tyrosine 5.20, and glutamic acid 0.53 per cent.

On pepsin digestion the histones, according to KOSSEL and PRINGLE[3] yield so-called histone-peptone, which also contains 25 per cent of the total nitrogen as arginine nitrogen. This histone-peptone differs from the protamines in not giving a precipitate with proteid in neutral or ammoniacal solution, but is precipitated in neutral reactions by sodium picrate. This property is used in its isolation.

According to KOSSEL the histones are probably intermediate bodies between the protamines and protein bodies on the demolition of the latter, and if this be true, then it is not to be expected that a sharp differentiation exists between histone and proteid, and for this reason it is hardly possible for the present to give a precise definition for the histones.

Protamines. In close relation to the proteins stands a group of substances, the protamines, discovered by MIESCHER, which are designated by KOSSEL as the simplest proteins or as the nucleus of the protein bodies. Thus far they have been found only in combination with nucleic acids in fish spermatozoa,[4] and the investigations of KOSSEL and WEISS[5] have shown that the material from which the protamines are

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[1] Amer. Journ. of Physiol, **19** and **23**.

[2] Zeitschr. f. physiol. Chem., **41**.

[3] *Ibid.*, **49**.

[4] Nelson, Arch. f. exp. Path. u. Pharm., **59**, has recently shown that the body called by him thymamine and prepared from the thymus glands, is a protamine, still he has not given sufficient evidence of the protamine nature of the substance.

[5] Zeitschr. f. physiol. Chem., **52**.

formed, at least in the salmon, is the muscle proteid. The question has been raised whether the protamines are true proteids or not, and whether it would not be more correct to consider them as cleavage products of proteid, or as fractions thereof. According to the generally accepted view we will treat them as true proteids.

Protamine was discovered by MIESCHER[1] in salmon spermatozoa. Later KOSSEL and his pupils isolated and studied similar bases from the spermatozoa of herring, sturgeon, mackerel, and other fishes. As all these bases are not identical, KOSSEL uses the name protamines to designate the group, and calls the individual protamines according to their origin *salmine, clupeine, scombrine, sturine, cyprinine, cyclopterine, crenilabrine* etc.

They differ materially from the proteins by the fact that they yield chiefly diamino-acids (always abundant arginine) as cleavage products, and only a small amount of monamino-acids. They are strongly basic substances rich in nitrogen (about 30 per cent or more) and have high molecular weight.

The percentage composition of these bodies has not been satisfactorily determined. As probable formulæ we have for salmine $C_{32}H_{54}N_{18}O_4$ (MIESCHER, SCHMIDEBERG, NELSON), or $C_{30}H_{57}H_{17}O_6$ (KOSSEL and GOTO), for clupeine $C_{30}H_{62}N_{14}C_9$, and for sturine $C_{36}H_{69}H_{19}O_7$ (KOSSEL) or $C_{34}H_{71}N_{17}O_9$ (GOTO), or according to MALENÜCK $C_{27}H_{55}H_{13}O_7$ for sturine from Accipenser Guldenstädtii. On boiling with dilute mineral acids as also by tryptic digestion, the protamines first yield peptone-like substances called *protones*, from which simple products (amino-acids) are derived on further cleavage. All protamines yield arginine, the four protamines salmine, clupeine, cyclopterine, and sturine, yielding 87.4, 82.2, 62.5, and 58.2 per cent respectively. In the three protamines salmine, clupeine and scombrine the arginine nitrogen, according to KOSSEL and PRINGLE[2], amounts to about 89 per cent of the total nitrogen. Sturine yields besides this the two hexone bases lysine, 12 per cent, and histidine, 12.9 per cent. Histidine has not been found in any other pro-

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[1] In regard to protamines, see Miescher, Histochemische und Physiologische Arbeiten, Leipzig, 1897; Piccard, Ber. d. deutsch. chem. Gesellsch., **7**; Schmiedeberg, Arch. f. exp. Path. u. Pharm., **37**; Kossel, Zeitschr. f. physiol. Chem., **22** (Ueber die basischen Stoffe des Zellkerns), **25**, 165 and 190, **26**, **40**, **44**, and **69**; and Sitzungsber. der Gesellsch. zur Beförd. der ges. Naturwiss. zu Marburg, 1897; Berl. klin. Wochenschr., 1904; Kossel and Mathews, Zeitschr. f. physiol. Chem., **23** and **25**; Kossel and Kutscher, *ibid.*, **31**; Goto, *ibid.*, **37**; Kurajeff, *ibid.*, **32**; Morkowin, *ibid.*, **28**; Kossel and Dakin, *ibid.*, **40**, **41**, and **44**; Malenück, *ibid.*, **57**; Pringle, *ibid.*, **49**; Kennaway, *ibid.*, **72**; Cameron, *ibid.*, **76**; F. Weiss, **59**, **60** and **78**; Nelson, Arch. f. exp. Path. u. Pharm., **59**.

[2] Zeitschr. f. physiol. Chem., **53**.

tamine. The carp protamine, cyprinine, occurs in two different modifications, namely, α- and β-cyprinine. The α-cyprinine yields only little arginine, 4.9 per cent, but the lysine content is pronounced, 28.8 per cent. Of the total nitrogen 30.3 per cent exists as lysine. KOSSEL and DAKIN have obtained from salmine the following cleavage products, namely, arginine 87.4, serine 7.8, aminovaleric acid 4.3, and α-pyrrolidine-carboxylic (proline) acid 11 per cent, and according to them the salmine contains about 10 mol. arginine, 2 mol. serine, 1 mol. aminovaleric acid, and 2 mol. proline. Scombrine contains only arginine, alanine, and proline. According to KOSSEL, every protamine contains only 2 or 3 monamino-acids (clupeine contains 4) and for every 2 molecules of arginine cnly 1 molecule of monamino-acid occurs. The above-mentioned protones (of the salmine group) are symmetrically constituted diarginides with a monamino-acid—for example diarginylserine, diarginylproline etc., —and these diarginides are united together forming the protamine. Thus according to KOSSEL in clupeine we can accept the presence of diarginylalanine, diarginylserine, diarginylproline, and diarginylvaline (KOSSEL and PRINGLE).

The following summary according to KOSSEL gives a view of the cleavage products of the protamines thus far investigated:

	Scombrine.	Salmine.	Clupeine.	Sturine.	Cyclopterine.	α-Cyprinine.	β-Cyprinine.	Crenilabrine.
Alanine	+	0	+	+	?	?	?	?
Serine	0	+	+	0	?	?	?	?
Valine	0	+	+	0	?	+	+	?
Leucine	0	0	0	+	?	?	?	?
Arginine	+	+	+	+	+	+	+	+
Lysine	0	0	0	+	0	+	+	+
Histidine	0	0	0	+	0	0	0	0
Proline	+	+	+	0	?	?	?	?
Tyrosine	0	0	0	0	+	0	0	+
Tryptophane	0	0	0	0	+	0	0	0

Solutions of these bases in water are alkaline and have the property of giving precipitates with ammoniacal solutions of proteins or primary proteoses, but the researches of HUNTER [1] show that these precipitates are not histones, as generally considered. The salts with mineral acids are soluble in water, but insoluble in alcohol and ether. They are more or less readily precipitated by neutral salts (NaCl). Among the salts of the protamines, the sulphate, picrate, and the double-platinum chloride are the most important, and are used in the preparation of the protamines. The protamines are, like the proteins, levogyrate; but by the action of alkali the rotation is reduced or made to disappear, which according

[1] Zeitschr. f. physiol. Chem. 53.

to KOSSEL and WEISS [1] depends at least in part, to a racemisation of the hexone bases, especially arginine within the protamine molecule. They give the biuret test beautifully, but with the exception of cyclopterine, β-cyprinine and crenilabrine do not give MILLON'S reaction. The protamine salts are precipitated in neutral or even faintly alkaline solutions by phosphotungstic acid, picric acid, chromic acid, and alkali ferrocyanides.

The protamines are prepared, according to KOSSEL, by extracting the heads of the spermatozoa, which have previously been extracted with alcohol and ether, with dilute sulphuric acid (1–2 per cent), filtering, and precipitating with 4 vols. of alcohol. The sulphate may be purified by repeated solution in water and precipitation with alcohol, and if necessary, conversion into the picrate. For more details see the works of KOSSEL and MALENÜCK. The double-platinum salt is best suited for analysis and can be obtained, according to GOTO, by precipitating the methyl-alcohol solution of the protamine hydrochloride with platinum chloride. MIESCHER also precipitates the base as a double-platinum salt.

B. Albuminoids or Albumoids.

Under this name we collect into a special group all those protein bodies which cannot be placed in either of the other groups. Most and best studied of the bodies belonging to this group are important constituents of the animal skeleton or the cutaneous structure. Some are hardened secretions, and all occur as a rule in an insoluble state in the organism, and they are distinguished in most cases by a pronounced resistance to reagents which dissolve proteins, or to chemical reagents in general, and it is due to these external properties that they are put in a special group. From a purely chemical standpoint there is no reason why they should be separated from the true proteids in a special group. Most of the bodies belonging to the albuminoids have been given on page 92.

The Keratins. Keratin is the chief constituent of the horny structure of the epidermis, of hair, wool, of the nails, hoofs, horns, feathers, of tortoise shell, etc., etc. Keratin is also found as neurokeratin (KÜHNE) in the brain and nerves. The shell membrane of the hen's egg seems also to consist of keratin, and according to NEUMEISTER [2] the organic matrix of the eggshells of various vertebrate animals belongs in most cases to the keratin group.

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[1] Zeitschr. f. physiol. Chem., **59**, **60**, and **78**.

[2] Kühne and Ewald, Verh. d. naturhistor.-med. Vereins zu Heidelberg (N. F.), **1**; also Kühne and Chittenden, Zeitschr. f. Biologie, **26**; Neumeister, *ibid.*, **31**.

It seems that there exist a number of keratins, and these form a special group of bodies. This fact, together with the difficulty in isolating the keratin from the tissues in a pure condition without a partial decomposition, is sufficient explanation for the variation in the elementary composition given below. As examples the analyses of a few tissues rich in keratin and of keratins are given:[1]

	C	H	N	S	O	
Human hair.....	43.72	6.34	15.06	4.95	29.93	(Rutherford and Hawk)
Nail.....	51.00	6.94	17.51	2.80	21.75	(Mulder)
Neurokeratin....	56.11–58.45	7.26–8.02	11.46–14.32	1.63–2.24	.....	(Kühne)
Neurokeratin....	56.61	7.45	14.17	2.27	.....	(Argiris)
Horn (average)..	50.86	6.94	.....	3.20	.....	(Horbaczewski
Tortoise shell....	54.89	6.56	16.77	2.22	19.56	(Mulder)
Shell membrane..	49.78	6.64	16.43	4.25	22.50	(Lindvall)
Egg membrane.. (Scyllium)	53.92	7.33	15.08	1.44	.....	(Pregl)

Mohr[2] has determined the quantity of sulphur in various keratin substances. Sulphur is in great part in loose combination, and it is removed principally by the action of alkalies (as sulphides), or indeed in part by boiling with water. Combs of lead after long usage become black, and this is due to the action of the sulphur of the hair. On heating keratin with water in sealed tubes to a temperature of 150° C. or higher, it dissolves with the elimination of sulphureted hydrogen or mercaptan (Bauer), and the solution contains proteose-like substances (Krukenberg) called *atmidkeratin* and *atmidkeratose* by Bauer.[3] Keratin is dissolved by alkalies, especially on warming, producing besides alkali sulphides also proteose substances.

Besides the well-known cleavage products such as leucine, tyrosine, aspartic acid, glutamic acid, arginine, and lysine, Fischer and Dörpinghaus,[4] have found glycocoll, alanine, valine, proline, serine, phenylalanine, and pyrrolidone-carboxylic acid (secondary from glutamic acid) among the cleavage products of horn substances. Emmerling claims to have found cystine as a sulphurized cleavage product, but K. Mörner

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[1] Rutherford and Hawk, Journ. of biol. Chem., **3**; Mulder, Versuch einer allgem. physiol. Chem., Braunschweig, 1844–51; Kühne, Zeitschr. f. Biologie, **26**; Horbaczewski, see Drechsel in Ladenburg's Handwörterbuch. d. Chem., **3**; Lindvall, Maly's Jahresbericht, 1881; Argiris, Zeitschr. f. physiol. Chem., **54**; Pregl., *ibid.*, 56.

[2] Zeitschr. f. physiol. Chem., **20**.

[3] Krukenberg, Untersuch. über d. chem. Bau d. Eiweisskörper, Sitzungsber. d. Janaischen Gesellsch. f. Med. u. Naturwissensch., 1886; Bauer, Zeitschr. f. physiol. Chem., **35**.

[4] Zeitschr. f. physiol. Chem., **36**, which contains also the older literature.

was the first to prove positively the abundant occurrence of cystine in the cleavage products. MÖRNER obtained from ox horn, human hair, and the shell-membrane of the hen's egg 6.8, 13.92, and 7.62 per cent cystine calculated on the basis of the dry substance. BUCHTALA [1] obtained the following amounts of cystine from the respective keratin formations, namely, 12.98–14.53 per cent from human hair, 5.15 per cent from nails, 7.98 per cent from horsehair, 3.20 per cent from horse hoofs, 7.27 per cent from ox hair, 5.37 per cent from ox hoofs, 7.22 from pig bristles, 2.17 per cent from pig hoofs, 6.30 per cent from goose feathers, 2.14 per cent from chicken spurs, 1.88 per cent from the epidermis scales of chicken feet and 4.7 per cent from elephant epidermis. From the amount of sulphur split off by alkali, MÖRNER concludes that, at least in ox horn and human hair, all the sulphur exists as cystine. GALIMARD [2] was able to get only a qualitative test for cystine in the keratin of the adder eggs. SUTER, MÖRNER, and FRIEDMANN [3] have obtained α-thiolactic acid as a hydrolytic cleavage product of the keratin substances. The last-mentioned investigator was also able to detect thioglycolic acid in the cleavage products of wool.

The shell membrane of the hen's egg, and the eggshells of amphibians and certain fishes are, as above mentioned, ordinarily classified as keratins. These bodies among themselves, as well as on comparison with other keratins, show a marked difference in properties, this being very evident from the tabulation on page 115.

The large quantity of cystine in the keratins is considered as especially characteristic, and they differ in this regard from the other proteins. The shell membrane of the hen's egg behaves like a keratin in regard to the large amount of cystine contained, but differs essentially by the absence of tyrosine. It is remarkable that the egg membrane of the Selachii, which biologically is analogous with ovokeratin, differs from the typical keratins by the absence of cystine, while it contains, on the contrary, large amounts of tyrosine. The typical keratins differ among themselves in regard to composition, thus the keratin from the sheep hoofs contains 2 per cent phenylalanine, while this amino-acid is absent in the keratin of hair and feathers. It is difficult to say whether or not this is due to a difference in the purity of the bodies or not. The keratins investigated chemically, thus far, do not form a sufficient characteristic group.

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[1] Mörner, *ibid.*, **34** and **42**; Emmerling, Ref. in Chemiker Zeitung, 1894; Buchtala, Zeitschr. f. physiol. Chem., **52**, **69**, and **78**.

[2] Chem. Centralbl. II, 1905.

[3] Suter, Zeitschr. f. physiol. Chem., **20**; Mörner, *ibid.*, **42**; Friedmann, Hofmeister's Beiträge, **2**.

	Keratin from Horse-hair.[1]	Keratin from Sheep Wool.[4]	Keratin from Goose Feathers[5]	Keratin from Sheep Horn.[4]	Shell Membrane of the Hen's egg.[6]	Egg Membrane of *Scyllium stellare*.[8]	Tortoise Shell of *Chelone imbricata*.[9]
Glycocoll	4.7	0.58	2.6	0.45	3.9	2.6	19.36
Alanine	1.5	4.40	1.8	1.6	3.5	3.2	2.95
Valine	0.9	2.80	0.5	4.5	1.1	—	5.23
Leucine	7.1	11.5	8.0	15.3	7.4	5.8	3.26
Serine	0.6	0.1	0.4	1.1	—	—	—
Aspartic acid	0.3	2.3	1.1	2.5	1.1	2.3	—
Glutamic acid [10]	3.7	12.9	2.3	17.2	8.1	7.2	—
Cystine	7.98[2]	7.3	—	7.5	7.62[7]	?	5.19
Phenylalanine	0.0	—	0.0	1.9	—	3.3	1.08
Tyrosine	3.2	2.9	3.6	3.6	0.0	10.6	13.59
Proline	3.4	4.4	3.5	3.7	4.0	4.4	—
Histidine	0.61[3]	—	—	—	—	1.7	—
Arginine	4.45[3]	—	—	2.7	—	3.2	—
Lysine	1.12[3]	—	—	0.2	—	3.7	—

Bodies occur in the animal kingdom which form to a certain extent intermediate substances between coagulated protein and keratin. C. TH. MÖRNER [11] has detected such a body (*albumoid*) in the tracheal cartilage which forms a net-like trabecular tissue. This substance appears to be related to the keratins on account of its solubilities and the quantity of the sulphur (lead-blackening) it contains, while according to its solubility in gastric juice it must stand close to the proteins. Another substance, nearly like keratin, is the horny layer in the gizzard of birds. According to J. HEDENIUS this substance is insoluble in gastric or pancreatic juice, and acts quite like keratin. According to K. B. HOFMANN and PREGL,[12] who call this substance *koilin*, it does not yield any cystine on hydrolysis, or at least not a determinable quantity. According to others the quantity of cystine is very small. BUCHTALA [13] obtained only

[1] Abderhalden and Wells, Zeitschr. f. physiol. Chem., **46**.

[2] Buchtala, *ibid.*, **52**.

[3] Argiris, *ibid.*, **54**.

[4] Abderhalden and Voitinovici, *ibid.*, **52**.

[5] Abderhalden and Le Count, *ibid.*, **46**.

[6] Abderhalden and Ebstein, *ibid.*, **48**.

[7] Körner, *ibid.*, **34** and **42**.

[8] Pregl, *ibid.*, **56**.

[9] Buchtala, *ibid.*, **74**.

[10] Abderhalden and Fuchs, Zeitschr. f. physiol. Chem., **57**, have shown that the same variety of keratin, on ageing of the horn structure, becomes somewhat poorer in glutamic acid.

[11] See Maly's Jahresber., **18**.

[12] Hedenius, Skand. Arch. f. Physiol., **8**; Hofmann and Pregl, Zeitschr. f. physiol., Chem., **52**.

[13] Zeitschr. f. physiol. Chem., **69**.

a little more than 0.5 per cent pure crystalline cystine and on account of the low cystine content as well as for other reasons the koilin differs from the keratins.

Keratin is amorphous or takes the form of the tissues from which it was prepared. It is insoluble in water, alcohol, or ether. On heating with water to 150–200° C. it dissolves. It also dissolves gradually in caustic alkalies, especially on heating. It is not dissolved by artificial gastric juice or by trypsin solutions. Keratin gives the xanthoproteic reaction, as well as the reaction with MILLON'S reagent, although the latter is not always typical.

In the preparation of keratin a finely divided horny structure is treated first with boiling water, then consecutively with diluted acid, pepsin-hydrochloric acid, and alkaline trypsin solution, and, lastly, with water, alcohol, and ether.

Elastin occurs in the connective tissue of higher animals, sometimes in such large quantities that it forms a special tissue. It occurs most abundantly in the cervical ligament (ligamentum nuchæ).

Elastin used to be generally considered as a sulphur-free substance. According to the investigations of CHITTENDEN and HART, it is a question whether or not elastin contains sulphur, as it may have been removed by the action of the alkali in its preparation. H. SCHWARZ has been able by another method, to prepare an elastin containing sulphur, from the aorta, and this sulphur can be removed by the action of alkalies, without changing the properties of the elastin; and ZOJA, HEDIN, BERGH, and RICHARDS and GIES[1] have found that elastin contains sulphur. The most trustworthy analyses of elastin from the cervical ligament (Nos. 1 and 2) and from the aorta (No. 3) have given the following results, which compare well with each other:

	C	H	N	S	O	
1.	54.32	6.99	16.75	....	21.94	(HORBACZEWSKI[2])
2.	54.24	7.27	16.70	....	21.79	(CHITTENDEN and HART)
3.	53.95	7.03	16.67	0.38	.....	(H. SCHWARZ)

ZOJA found 0.276 per cent sulphur and 16.96 per cent nitrogen in elastin. HEDIN and BERGH found different quantities of nitrogen in aorta-elastin, depending upon whether HORBACZEWSKI'S or SCHWARZ'S method was used in its preparation. In the first case they found 15.44 per cent nitrogen and 0.55 per cent sulphur, and in the other 14.67 per

[1] Chittenden and Hart, Zeitschr. f. Biologie,' **25**; Schwarz, Zeitschr. f. physiol. Chem., **18**; Zoja, *ibid.*, **23**; Bergh, *ibid.*, **25**; Hedin, *ibid.*; Richards and Gies, Amer. Journ. of Physiol., **7**.

[2] Zeitschr. f. physiol. Chem., **6**.

cent nitrogen and 0.66 per cent sulphur. RICHARDS and GIES found 0.14 per cent sulphur and 16.87 per cent nitrogen in elastin. The question whether elastin is a unit body still remains open.

The quantity of hydrolytic cleavage products are given in the table on page 125. It is sufficient to here call attention to the fact that no aspartic acid and only very little glutamic acid have been found. The hexone bases have been obtained, but only in very small amounts, so that the basic nitrogen represents only 3.34 per cent of the total nitrogen (RICHARDS and GIES). From an elastin proteose, WECHSLER[1] obtained 1.86 per cent arginine, 0.5 per cent, histidine and 2.48 per cent lysine.

Indol and skatol have not been found on the putrefaction of elastin,[2] but SCHWARZ, on the contrary, obtained indol, skatol, benzene, and phenols on fusing aorta-elastin with caustic potash. On heating with water in closed vessels, on boiling with dilute acids, or by the action of proteolytic enzymes, the elastin dissolves and splits into two chief products, called by HORBACZEWSKI *hemielastin* and *elastinpeptone*. According to CHITTENDEN and HART, these products correspond to two proteoses designated by them *protoelastose* and *deuteroelastose*. The first is soluble in cold water and separates out on heating, and its solution is precipitated by mineral acid as well as by acetic acid and potassium ferrocyanide. The aqueous solution of the other does not become cloudy on heating, and is not precipitated by the above-mentioned reagents.

Pure elastin when dry is a yellowish-white powder; in the moist state it appears like yellowish-white threads or membranes. It is insoluble in water, alcohol, or ether, and shows a resistance toward the action of chemical reagents. It is not dissolved by strong caustic alkalies at the ordinary temperature and only slowly at the boiling temperature. It is very slowly attacked by cold concentrated sulphuric acid, but it is relatively easily dissolved on warming with strong nitric acid. Elastins of different origin act differently with cold concentrated hydrochloric acid; for instance, elastin from the aorta dissolves readily therein, while elastin from the ligamentum nuchæ, at least from old animals, dissolves with difficulty. Elastin is more readily dissolved by warm concentrated hydrochloric acid. It responds to the xanthoproteic reaction, and to that with MILLON'S reagent, but not to the ADAMKIEWICZ-HOPKINS reaction.

On account of its great resistance to chemical reagents, elastin may be prepared (best from the ligamentum nuchæ) in the following way: First boil with water, then with 1 per cent caustic potash, then again

[1] Zeitschr. f. physiol. Chem., **67**.

[2] See Wälchli, Journ. f. präkt. Chem. (N. F.), **17**.

with water, and lastly with acetic acid. The residue is treated with cold 5 per cent hydrochloric acid for twenty-four hours, carefully washed with water, boiled again with water, and then treated with alcohol and ether.

In regard to the methods used by SCHWARZ and by RICHARDS and GIES, which are somewhat different, we refer to the original publications.

Collagen, or gelatin-forming substance, occurs very extensively in vertebrates. The flesh of cephalopods is also said to contain collagen.[1] Collagen is the chief constituent of the fibrils of the connective tissue and (as ossein) of the organic substances of the bony structure. It also occurs in the cartilaginous tissues as chief constituent; but it is here mixed with other substances, producing what was formerly called chondrigen. Collagen from different tissues has not quite the same composition, and probably there are several varieties of collagen.

By continued boiling with water (more easily in the presence of a little acid) collagen is converted into gelatin. HOFMEISTER[2] found that gelatin on being heated to 130° C. is again transformed into collagen; and this last may be considered as the anhydride of gelatin. Collagen and gelatin have about the same composition.[3]

	C	H	N	S	O	
Collagen.....	50.75	6.47	17.86		24.92	(HOFMEISTER)
Gelatin (commercial)....	49.38	6.80	17.97	0.70	25.13	(CHITTENDEN)
Gelatin from tendons....	50.11	6.56	17.81	0.26	25.26	(VAN NAME)
Gelatin from ligaments...	50.49	6.71	17.90	0.57	24.33	(RICHARDS and GIES)
Fish glue (isinglass).....	48.69	6.76	17.68	—	—	(FAUST)

Gelatins of different origin show a somewhat variable composition, which seems to indicate the occurrence of different collagens. It is difficult to say whether the variable content of sulphur is due to a contamination with a substance rich in sulphur or to a splitting off of loosely combined sulphur during the purification. C. MÖRNER[4] has prepared a typical gelatin containing only 0.2 per cent of sulphur by a method which eliminated any possible changes due to reagents.

SADIKOFF[5] has prepared gelatins by various methods from tendons and from cartilage. Those from tendons, some of which were prepared after previous tryptic digestion, some after treatment with 0.25 per cent caustic potash, and some after treatment with sodium hydroxide and then carbonate, showed

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[1] Hoppe-Seyler, Physiol. Chem., p. 97.

[2] Zeitschr. f. physiol. Chem., **2**.

[3] Hofmeister, l. c.; Chittenden and Solley, Journ. of Physiol., **12**; van Name, Journ. of Exper. Med., **2**; Richards and Gies, Amer. Journ. of Physiol., **8**; Faust, Arch. f. exp. Path. u. Pharm., **41**.

[4] Zeitschr. f. physiol. Chem., **28**.

[5] *Ibid.*, **39** and **41**.

somewhat different physical properties among each other, but had about the same elementary composition, with 0.34–0.53 per cent sulphur. SADIKOFF seems to think that the gelatins prepared up to this time were perhaps not unit bodies but were possibly mixtures. The bodies prepared by SADIKOFF from cartilage he calls *gluteins*, because they were essentially different from the other gelatins or glutins. They were poorer in carbon and nitrogen, 17.17 to 17.87 per cent, but somewhat richer in sulphur, 0.53–0.718 per cent, than the tendon glutin. The gluteins differ also from the glutins in that on boiling with a mineral acid they have a faint reducing action, and also in that they give a color reaction with phloroglucin-hydrochloric acid which is probably due to contamination. The glutins differ from the gluteins by a different behavior with certain salts.

The decomposition products of the collagens are the same as those of the gelatins and will be found in the table on page 175. Of special mention is the fact that gelatin contains no tyrosine and tryptophane but does yield considerable glycocoll. This latter substance has, because of its sweet taste, been called gelatin sugar. SKRAUP [1] has obtained on the hydrolytic cleavage of gelatin a crystalline acid having the formula $C_{12}H_{25}N_5O_{10}$, which he calls *glutinic acid*. Gelatin yields considerable basic nitrogen, according to HAUSMANN,[2] 35.83 per cent of the total nitrogen. It also yields considerable arginine (9.3 per cent), lysine 5–6 per cent, but only little histidine (0.4 per cent). The aromatic group in gelatin is therefore, as directly shown by FISCHER and also by SPIRO,[3] represented by phenylalanine.

Collagen is insoluble in water, salt solutions, and dilute acids and alkalies, but it swells up in dilute acids. By continued boiling with water it is converted into gelatin. Various collagens are converted into gelatin with varying readiness; the formation of gelatin occurs also from difficultly soluble collagens by continuous boiling with water. Collagen is dissolved by the gastric juice and also by the pancreatic juice (trypsin solution) when it has previously been treated with acid or heated with water above 70° C.[4] By the action of ferrous sulphate, corrosive sublimate, or tannic acid, collagen shrinks greatly. Collagen treated by these bodies does not putrefy, and tannic acid is therefore of great importance in the preparation of leather.

Gelatin or glutin is colorless, amorphous, and transparent in thin layers. It swells in cold water without dissolving. It dissolves in warm water, forming a sticky liquid, which solidifies on cooling when sufficiently concentrated. As PAULI and RONA [5] have shown, various bodies may have a different influence upon the gelatinization-point of a gelatin

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[1] Monatshefte f. Chem., **26**.

[2] Zeitschr. f. physiol. Chem., **27**.

[3] Fischer, Levene and Aders, Zeitschr. f. physiol. Chem., **35**; Spiro, Hofmeister's Beiträge, **1**.

[4] Kühne and Ewald, Verh. d. Naturhist. Med. Vereins in Heidelberg, 1877, **1**.

[5] Hofmeister's Beiträge, **2**.

solution; thus certain substances such as sulphates, citrates, acetates, and glycerin may accelerate, while the chlorides, chlorates, bromides, alcohol, and urea retard, this power.

Gelatin solutions are not precipitated on boiling, or by mineral acids, acetic acid, alum, basic lead acetate, or metallic salts in general. A gelatin solution acidified with acetic acid may be precipitated by potassium ferrocyanide on carefully adding the reagent. Gelatin solutions are precipitated by tannic acid in the presence of salt, and according to TRUNKEL [1] completely if the gelatin and tannic acid are in the proportion 1:0.7. According to him the precipitation is not due to a chemical combination but to an adsorption phenomenon. Solutions of gelatin in water are also precipitated by acetic acid and common salt in substance; mercuric chloride in the presence of HCl and NaCl; by metaphosphoric acid and phosphomolybdic acid in the presence of acid; and lastly also by alcohol, especially when neutral salts are present. Gelatin solutions do not diffuse. Gelatin gives the biuret reaction, but not ADAMKIEWICZ-HOPKINS reaction. It gives MILLON'S reaction and the xanthoproteic reaction so faintly that they probably occur from impurities consisting of proteids. According to C. MÖRNER, pure gelatin gives a beautiful MILLON'S reaction, if not too much reagent is added. In the other case no reaction or only a faint one is obtained.

By continued boiling with water gelatin is converted into a non-gelatinizing modification called β-glutin by NASSE. According to NASSE and KRÜGER the specific rotatory power is hereby reduced from $-167.5°$ to about $-136°$.[2] According to TRUNKEL, who has especially studied the rotation behavior of gelatin, the rotation of β-glutin is less than the ordinary α-glutin. On prolonged boiling with water, especially in the presence of dilute acids, also in the gastric or tryptic digestion, the gelatin is transformed into gelatin proteoses, so-called *gelatoses* and *gelatin peptones*, which diffuse more or less readily.

According to HOFMEISTER two new substances, *semiglutin* and *hemicollin*, are formed. The former is insoluble in alcohol of 70–80 per cent and is precipitated by platinum chloride. The latter, which is not precipitated by platinum chloride, is soluble in alcohol. CHITTENDEN and SOLLEY [3] have obtained in the peptic and tryptic digestion a *proto-* and a *deutero-gelatose*, besides a true peptone. The elementary composition of these gelatoses does not essentially differ from that of the gelatin.

PAAL [4] has prepared gelatin-peptone hydrochlorides from gelatin by the action of dilute hydrochloric acid. These salts are partly soluble in ethyl and

[1] Bioch. Zeitschr., **26**.

[2] Nasse and Krüger, Maly's Jahresber., **19**, p. 29. In regard to the rotation of β-glutin, see Framm, Pflüger's Arch., **68**; Trunkel, l. c.

[3] Hofmeister, l. c.; Chittenden and Solley, l. c.

[4] Ber. d. deutsch. chem. Gesellsch., **25**.

methyl alcohol, and partly insoluble therein. The peptones obtained from these salts contain less carbon and more hydrogen than the gelatin from which they originated, showing that hydration has taken place. The molecular weight of the gelatin peptone as determined by PAAL, by RAOULT'S cryoscopic method, was 200 to 352, while that for gelatin was 878 to 950. The gelatin peptones isolated by SIEGFRIED and his pupils which will be discussed below, are of great interest.

Collagen (contaminated with mucoid) may be obtained from bones by extracting them with hydrochloric acid (which dissolves the earthy phosphates) and then carefully washing the acid out with water. It may be obtained from tendons by extracting with lime-water or dilute alkali (which dissolve the proteids and mucin), and then thoroughly washing with water. Gelatin is obtained by boiling collagen with water. The finest commercial gelatin always contains a little proteid, which may be removed by allowing the finely divided gelatin to swell up in water and thoroughly extracting with large quantities of fresh water. Then dissolve in warm water and precipitate with alcohol.

Collagen may also be purified from proteids, as suggested by VAN NAME, by digesting with an alkaline trypsin solution or by extracting the gelatin for many days with 1–5 p. m. caustic potash, as suggested by C. MÖRNER. The typical properties of gelatin are not changed by this.

Chondrin or cartilage gelatin is only a mixture of gelatin with the specific constituents of the cartilage and their transformation products.

Reticulin. The reticular tissues of the lymphatic glands contain a variety of fibers which have also been found, by MALL in the spleen, intestinal mucosa, liver, kidneys, and lungs. These fibers consist of a special substance, reticulin, investigated by SIEGFRIED.[1]

Reticulin has the following composition: C 52.88; H 6.97; N 15.63; S 1.88; P 0.34; ash 2.27 per cent. The phosphorus occurs in organic combination. It yields no tyrosine on cleavage with hydrochloric acid. It yields, on the contrary, sulphureted hydrogen, ammonia, lysine, arginine, and valine. On continued boiling with water, or more readily with dilute alkalies, reticulin is converted into a body which is precipitated by acetic acid, and at the same time phosphorus is split off.

Reticulin is insoluble in water, alcohol, ether, lime-water, sodium carbonate, and dilute mineral acids. It is dissolved, after several weeks, on standing with caustic soda at the ordinary temperature. Pepsin-hydrochloric acid or trypsin does not dissolve it. Reticulin responds to the biuret, xanthoproteic, and ADAMKIEWICZ-HOPKINS reactions, but not to MILLON'S reagent.

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[1] Mall, Abhandl. d. math.-phys. Klasse d. Kgl. sächs. Gesellsch. d. Wiss., 1891; Siegfried, Ueber die chem. Eigensch. der retikulirten Gewebe, Habil.-Schrift, Leipzig, 1892.

According to TEBB reticulin is only a somewhat changed, impure collagen but this is disputed by SIEGFRIED.[1]

It may be prepared as follows, according to SIEGFRIED: Digest intestinal mucosa with trypsin and alkali. Wash the residue, extract with ether, and digest again with trypsin and then treat with alcohol and ether. On careful boiling with water the collagen present either as contamination or as a combination with recticulin is removed. The thoroughly boiled residue consists of reticulin.

Ichthylepidin is an organic compound, so-called by C. MÖRNER,[2] which occurs with collagen in fish-scales and forms about one-fifth of their organic substance. This compound, with 15.9 per cent nitrogen and 1.1 per cent sulphur, stands on account of its properties rather close to elastin. It is insoluble in cold and hot water, as well as in dilute acids and alkalies at the ordinary temperature. On boiling with these it dissolves. Pepsin-hydrochloric acid, as well as an alkaline trypsin solution, also dissolves it. It responds beautifully to MILLON's reagent, the xanthoproteic reaction, and the biuret test. At least a part of the sulphur is split off by the action of alkali. Ichthylepidin stands very close to elastin in regard to its solubilities; but it differs essentially in composition as it is markedly poorer in glycocoll, but much richer in proline and glutamic acid (ABDERHALDEN and VOITINOVICI [3]).

As **skeletins**, KRUKENBERG [4] has designated a number of nitrogenized substances which form the skeletal tissue of various classes of invertebrates. These substances are *chitin*, *spongin*, *conchiolin*, *byssus*, *cornein*, and *crude silk* (*fibroin* and *sericin*). Of these, chitin does not belong to the protein substances, and silk is hardly to be classed as a skeletin. Only those so-called skeletins will be discussed that actually belong to the protein group, and chitin will be discussed in another chapter.

The elementary composition of certain of the bodies belonging to this group is as follows: [5]

	C	H	N	S	
Conchiolin (from the shells of pinna) ...	52.70	6.54	16.60	0.85	(WETZEL)
Spongin	46.50	6.30	16.20	0.50	(CROOKEWITT)
"	48.75	6.35	16.40	....	(POSSELT)
Cornein	48.96	5.90	16.81	....	(KRUKENBERG)
Fibroin	48.23	6.27	18.31	....	(CRAMER)
"	48.30	6.50	19.20	....	(VIGNON)
Sericin	44.32	6.18	18.30	....	(CRAMER)
"	44.50	6.32	17.14	....	(BONDI)

[1] Tebb, Journ. of Physiol., **27**; Siegfried, *ibid.*, **28**.

[2] Zeitschr. f. physiol. Chem., **24** and **37**. See also Green and Tower, *ibid.*, **35**.

[3] Zeitschr. f. physiol. Chem., **52**, p. 368.

[4] Grundzüge einer vergl. Physiol. d. thier. Gerüstsubst. Heidelberg, 1885.

[5] Krukenberg, Ber. d. d. chem. Gesellsch., **17** and **18**, and Zeitschr. f. Biologie, **22**; Croockewitt, Annal. d. Chem. u. Pharm., **48**; Posselt, *ibid.*, **45**; Cramer, Journ. f. prakt. Chem., **96**; Vignon, Compt. rend., **115**; Wetzel, Zeitschr. f. physiol. Chem., **29** and Centralbl. f. Physiol., **13**, 113; Bondi, Zeitschr. f. physiol. Chem., **34**.

Spongin forms the chief mass of the ordinary sponge. It dissolves with difficulty in concentrated mineral acids but dissolves with readiness in caustic alkalies. It does not give the MILLON reaction or ADAMKIEWICZ'S. It gives no gelatin. On hydrolysis spongin yields considerable glycocoll 13.9 per cent, glutamic acid 18.1 per cent, leucine 7.5 per cent, proline 6.3 per cent, lysine 3–4 per cent, and arginine 5–6 per cent.[1] Tyrosine and phenylalanine could not be detected. After HUNDESHAGEN had shown the occurrence of iodine and bromine in organic combination in different sponges and designated the albumoid containing iodine, *iodospongin*, HARNACK [2] later isolated from the ordinary sponge, by cleavage with mineral acids, an iodospongin which contained about 9 per cent iodine and 4.5 per cent sulphur. STRAUSS [3] has obtained *sponginoses* of various kinds from spongin by dilute acids. The heterosponginose contained the greater part of the iodine and sulphur, while the deuterosponginose contained the carbohydrate groups. Iodospongin is considered as a derivative of the heterosponginose. **Conchiolin** is found in the shells of mussels and snails and also in the eggshells of these animals. It yields, according to WETZEL,[4] glycocoll, leucine, and abundance of tyrosine. The quantity of diamino-nitrogen amounts to 8.7 per cent and the amide nitrogen 3.47 per cent (from the shell of pinna). The **Byssus** contains a substance, closely related to conchiolin, which is soluble with difficulty. According to ABDERHALDEN [5] it yields considerable glycocoll and tyrosine and also alanine, aspartic acid and very large amounts of proline.

Cornein is the name given to the substance of the axial system of certain Anthozoa. The substance occurring in the groups of Gorgonia and Antipathes has been called *gorgonin* by C. MÖRNER [6] and differs from the *pennatulin* of the Pennatulideæ by the latter being readily soluble in pepsin-hydrochloric acid. The cleavage products have not been carefully studied; one of the crystalline products, called *cornicrystalline* by KRUKENBERG, is nothing but iodine crystals, as shown by MÖRNER. After DRECHSEL [7] found nearly 8 per cent iodine in the dry substance of the axial system of the Gorgonia Cavolini, C. MÖRNER showed that in the Anthozoa in general the organic skeletal substance contains halogens in organic combination. Iodine was found in all varieties, and indeed in amounts from traces up to 7 per cent. Bromine was found, with the exception of two Antipathes, in amounts of 0.25 to 4 per cent, while chlorine, which was never absent, occurred as a few tenths per cent. The halogens occur in the organic skeletal substance as gorgonin and pennatulin.

DRECHSEL obtained leucine, tyrosine, lysine, ammonia and an iodized amino-acid, *iodogorgonic acid*, as cleavage products of gorgonin. This last

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[1] Abderhalden and Strauss, Zeitschr. f. physiol. Chem., 48; Kossel and Kutscher, *ibid.*, 31, 205.

[2] Zeitschr. f. physiol. Chem., 24; Hundeshagen, Maly's Jahresber., 25, 394; see also L. Scott, Biochem. Zeitschr., 1.

[3] Biochem. Centralbl., 3.

[4] Zeitschr. f. physiol. Chem. 29, and Centralbl. f. Physiol., 13, 113.

[5] Zeitschr. f. physiol. Chem., 55.

[6] Zeitschr. f. physiol. Chem., 51 and 55.

[7] Zeitschr. f. Biol., 33.

is identical with 3-5 di-iodo-tyrosine, $HOI_2C_6H_2.CH_2.CHNH_2COOH$, synthetically prepared by WHEELER and JAMIESON.[1] On acid cleavage of gorgonin, HENZE[2] obtained the three hexone bases, abundant tyrosine and very little leucine. On cleavage with barium hydroxide he obtained only lysine, besides tyrosine and glycocoll in larger amounts.

Fibroin and **sericin** are the two chief constituents of raw silk. By the action of boiling water the sericin (silk gelatin) dissolves and can be obtained by a method suggested by BONDI,[3] while the more difficultly soluble fibroin remains undissolved in the shape of the original fiber. The sericin, whose sufficiently concentrated hot solution gelatinizes on cooling, is precipitated by mineral acids, several metallic salts, and by acetic acid and potassium ferrocyanide. The spider silk investigated by FISCHER[4] yielded fibroin but not sericin.

ABDERHALDEN and his collaborators[5] have investigated a great number of varieties of silk and found sericin in varying amounts (15 to 28 per cent). The composition of the various kinds of silk is characterized, especially, by a varying amount of glycocoll and in this regard we can differentiate between two chief groups. The one group is, like the Italian silk, very rich in glycocoll while the other group, like the Tussah silk, contains a much smaller quantity of glycocoll.

Sericin, whose proper concentrated warm solution gelatinizes on cooling, is precipitated by mineral acids and several metallic salts and by acetic acid and potassium ferrocyanide. In regard to the products of hydrolysis it differs very essentially from fibroin by being much poorer in glycocoll, alanine and tyrosine.

Fibroin is soluble in concentrated acids and alkalies and reprecipitable (in a modified form) on neutralization. It gives the biuret test and MILLON'S and ADAMKIEWICZ-HOPKIN'S reactions, the last but faintly. Fibroin has an especially great interest because of the hydrolyses performed by FISCHER and his co-workers, and especially by the finding of the previously mentioned polypeptides by these workers. Of the cleavage products which characterize fibroin we must mention the large amount of glycocoll, alanine and tyrosine, and the very small amounts of hexone bases, besides the almost complete absence of monamino-dicarboxylic acids. The quantity of the hydrolytic cleavage products of the three silk substances, in so far as they have been investigated, are given in the following table, which also includes the results for elastin, gelatin, and

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[1] Wheeler and Jamieson, Amer. Chem. Journ., 33; Wheeler, *ibid.*, 38.
[2] Henze, Zeitschr. f. physiol. Chem., 38 and 51.
[3] Zeitschr. f. physiol Chem., 34.
[4] *Ibid.*, 53.
[5] See Zeitschr. f. physiol. Chem., 59, 61, 62, 64, 71, 74, 80.

koilin. The fibroin **A** came from ordinary silk; fibroin **B** and the sericin originated from Indian Tussah silk.

	Elastin.[1]	Gelatin.[2]	Koilin.[4]	Fibroin *A*[1]	Fibroin *B*[6]	Sericin.[7]	SpiderSilk[8]
Glycocoll	25.75	19.25	1.2	36.0	9.5	1.5	35.13
Alanine	6.6	3.0	5.8	21.0	24.0	9.8	23.4
Valine	1.0	—	—	—	—	—	—
Leucine	21.1	9.2[3]	13.2	1.5	1.5	4.8	1.76
Serine	—	0.4	—	1.6	2.0	5.4	—
Aspartic acid	—	1.2[3]	2.3	—	2.5	2.8	—
Glutamic acid	0.8	16.8[3]	5.2	—	1.0	1.8	11.70
Cystine	—	—	0.7[10]	—	—	—	—
Phenylalanine	3.9	1.0[3]	2.3	1.5	0.6	0.3	—
Tyrosine	0.34	—	5.4	10.5	9.2	1.0	8.20
Proline	1.7	7.7	5.5	—	1.0	3.0	3.68
Oxyproline	—	6.4	—	—	—	—	—
Histidine	—	0.4	0.03[5]	—	—	—	5.24[9]
Arginine	0.3	9.3	3.60[5]	1.0	—	—	
Lysine	—	5.6	1.64[5]	—	—	—	

C. Cleavage Products of Simple Proteins.

On the hydrolysis of proteins by the aid of acids, alkalies or by enzymes, cleavage products are obtained which represent various intermediary steps between the native proteins on one side and the simple cleavage products, the amino-acids, on the other side. Among these products we have for a long time known two chief groups which still retain, to a high degree, their protein character, namely, the albuminates and the proteoses (and peptones).

1. Albuminates.

Alkali and Acid Albuminates. The native proteins are modified by the action of sufficiently strong acids or alkalies. By the action of alkalies all native albuminous bodies are converted, with the elimination of nitrogen, or by the action of stronger alkali, with the extraction of sulphur also, into a new modification, called alkali albuminate. If caustic alkali in substance or in strong solution be allowed to act on a

[1] Cited from Abderhalden's Lehrbuch d. physiol. Chem., 1909.
[2] Cohnheim, Chemie d. Eiweisskörper 3 d. Aufl.
[3] Skraup and Biehler, Monatsh. f. Chem., **30**.
[4] K. B. Hoffmann and Pergl. Zeitschr. f. physiol. Chem., **52**.
[5] v. Knaffl-Lenz, *ibid.*, **52**.
[6] Abderhalden and Spack, *ibid.*, **62**.
[7] Strauch, *ibid.*, **71**.
[8] E. Fischer, *ibid.*, **53**.
[9] Calculated as arginine.
[10] This figure is somewhat uncertain.

concentrated proteid solution, such as blood-serum or egg-albumin, the alkali albuminate may be obtained as a solid jelly which dissolves in water on heating, and which is called "LIEBERKÜHN'S solid alkali albuminate." By the action of dilute caustic alkali solutions on dilute proteid solutions we have alkali albuminates formed slowly at the ordinary temperature, but more rapidly on heating. These solutions may vary with the nature of the proteid acted upon, and also with the intensity of the action of the alkali, but still they have certain reactions in common.

If proteid is dissolved in an excess of concentrated hydrochloric acid, or if we digest a proteid solution acidified with 1–2 p. m. hydrochloric acid in the thermostat, or digest the proteid for a short time with pepsin-hydrochloric acid, we obtain new modifications of proteid which may show somewhat varying properties, but have certain reactions in common. These modifications, which may be obtained in a solid gelatinous condition on sufficient concentration, are called acid albuminates or acid albumins, and sometimes syntonin, though we perfer to apply the term syntonin to the acid albuminate, which is obtained by extracting muscles with hydrochloric acid of 1 p. m.

The alkali and acid albuminates have the following reactions in common: They are almost insoluble in water and dilute common-salt solution (see page 104), but they dissolve readily in water on the addition of a very small quantity of acid or alkali. Such a solution as nearly neutral as possible does not coagulate on boiling but is precipitated at the normal temperature on neutralizing the solvent by an alkali or an acid. A solution of an alkali or acid albuminate in acid is easily precipitated on saturating with NaCl, but a solution in alkali is precipitated with difficulty or not at all, according to the amount of alkali it contains. Mineral acids in excess precipitate solutions of acid as well as alkali albuminates. The nearly neutral solutions of these bodies are also precipitated by many metallic salts.

Notwithstanding this agreement in the reactions, the acid and alkali albuminates are essentially different, for by dissolving an alkali albuminate in some acid no acid, albuminate solution is obtained, nor is an alkali albuminate formed on dissolving an acid albuminate in water by the aid of a little alkali. In the first case we obtain a combination of the alkali albuminate and the acid, soluble in water, and in the other case a soluble combination of the acid albuminate with the alkali added. The chemical process in the modification of proteids with an acid is essentially different from the modification with an alkali, hence the products are of a different kind. The alkali albuminates are relatively strong acids. They may be dissolved in water with the aid of $CaCO_3$, with the elimination of CO_2, which does not occur with typical acid albuminates, and they show in opposition to the acid albuminates also other variations

which stand in connection with their strongly marked acid nature. Dilute solutions of alkalies act more energetically on proteids than do acids of corresponding concentration. In the first case a part of the nitrogen and often also the sulphur, is split off, and from this property we may obtain an alkali albuminate by the action of an alkali upon an acid albuminate; but we cannot obtain an acid albuminate by the reverse reaction (K. MÖRNER [1]). This does not exclude the possibility that, by a more severe acid treatment, products can be obtained which perhaps correspond to those products obtained by a more mild alkali treatment.

The preparation of the albuminates has been given above. The corresponding albuminate obtained by the action of alkalies or acids upon a proteid solution may be precipitated by neutralizing with acid or alkali. The washed precipitate is dissolved in water by the aid of a little alkali or acid, and again precipitated by neutralizing the solvent. If this precipitate, which has been washed in water, is treated with alcohol and ether, the albuminate will be obtained in a pure form.

In the preparation of acid as well as of alkali albuminates, proteoses and the closely related albuminates are formed. The "*alkali albumose*" obtained by MAAS [2] belongs to this class. The *lysalbinic acid* and *protalbinic acid* obtained by PAAL [3] from ovalbumin are likewise alkali albuminates. These have been carefully studied by SKRAUP and his co-workers.[4]

Desaminoalbuminic acid is an alkali albuminate which SCHMIEDEBERG [5] obtained by the action of such weak alkali that a part of the nitrogen was evolved but the quantity of sulphur remained the same. The proteid combination obtained by BLUM [6] by the action of formol on proteid and called by him *protogen*, has similarities with the alkali albuminates in regard to solubilities and precipitation, but is not identical therewith.

2. Proteoses and Peptones.

Peptones were formerly designated as the final products of the decomposition of protein bodies by means of proteolytic enzymes in so far as these final products are still true proteins, while the intermediate products produced in the peptonization of proteins, in so far as they are not substances similar to albuminates, were designated as proteoses (albumoses, or propeptones). Proteoses and peptones may also be produced by the hydrolytic decomposition of the proteins with acids

[1] Pflüger's Arch., 17.

[2] Zeitschr. f. physiol. Chem., 30.

[3] Ber. d. d. chem. Gesellsch., 35.

[4] Hummelberger, Lampel and Woeber, Monatsh. f. Chem., 30.

[5] Arch. f. exp. Path. u. Pharm., 39.

[6] Blum, Zeitschr. f. physiol. Chem., 22. The older investigations of Loew may be found in Maly's Jahresber., 1888. On the action of formaldehyde see also Benedicenti, Arch. f. (Anat. u.) Physiol., 1897; S. Schwarz, Zeitschr, f. physiol. Chem., 30; Bliss and Novy, Journ. of Exper. Med., 4.

or alkalies, and by the putrefaction of the same. They may also be formed in very small quantities, as by-products, in the investigations of animal fluids and tissues, and the question as to the extent to which these exist preformed under physiological conditions requires very careful investigation.

Between the peptone, which represents the final cleavage product, and the proteose, which stands closest to the original protein, we have undoubtedly a series of intermediate products. Under such circumstances it is a difficult problem to try to draw a sharp line between the peptone and the proteose group, and it is just as difficult to define our conception of peptones and proteoses in an exact and satisfactory manner.

In the past we used to consider the peptones as the end products in the hydrolysis, they still being true proteins, but we must call attention to the fact that since that time we have learned of polypeptide-like cleavage products of the proteins, and also that polypeptides have been prepared synthetically. With this in mind it is not possible to say what we understand by the conception true proteid, and also that possibly there exists a large number of intermediary steps between the original modified proteid and the simplest cleavage products. There is no doubt that those bodies which have been called proteoses and peptones are chiefly mixtures; and the question has been proposed by ABDERHALDEN [1] whether it is not best to drop the conception of proteoses and to call all products precipitable by ammonium sulphate, etc., and previously described as proteoses, peptones.

Although there is much in favor of such a proposition, still on account of the great importance which the conception of the proteoses has generally received, it is probably too early to drop the question of proteoses entirely from a text-book, and we will therefore, as in the past editions, discuss the historical development of the proteoses and peptones in the ordinary sense.

The **proteoses** (or **albumoses**) used to be considered as those protein bodies whose neutral or faintly acid solutions do not coagulate on boiling and which, to distinguish them from peptones, were characterized chiefly by the following properties: The watery solutions are precipitated at the ordinary temperature by nitric acid, as well as by acetic acid and potassium ferrocyanide, and this precipitate has the peculiarity of disappearing on heating and reappearing on cooling. If a proteose solution is saturated with NaCl in substance, the proteose is partly precipitated in neutral solutions, but on the addition of acid saturated with salt it is more completely precipitated. This precipitate, which dissolves on warming, is a combination of the proteose with the acid.

[1] Oppenheimer's Handb. der Biochem., Bd. 1, 1908.

We formerly designated as **peptones** those protein bodies which are readily soluble in water and which are not coagulated by heat, whose solutions are precipitated neither by nitric acid, nor by acetic acid and potassium ferrocyanide, nor by NaCl and acid.

The reactions and properties which the proteoses and peptones have in common were formerly considered as the following: They all give the color reactions of the proteins, but with the biuret test they give a more beautiful red color than the ordinary proteins. They are precipitated by ammoniacal lead acetate, by mercuric chloride, tannic, phosphotungstic, and phosphomolybdic acids, by potassium-mercuric iodide and hydrochloric acid, and also by picric acid. They are precipitated but not coagulated by alcohol, that is, the precipitate obtained is soluble in water even after being in contact with alcohol for a long time. The proteoses and peptones also have a greater diffusive power than native proteins, and the diffusive power is greater the nearer the questionable substance stands to the final product, the now so-called true peptone.

These old views have gradually undergone an essential change. After HEYNSIUS'[1] observation that ammonium sulphate was a general precipitant for proteins, and for peptones in the old sense, KÜHNE and his pupils[2] proposed this salt as a means of separating proteoses and peptones. Those products of digestion which separate on saturating their solution with ammonium sulphate, or can indeed be salted out at all, are considered, by KÜHNE and also by most of the modern investigators, as proteoses, while those which remain in solution are called peptones or true peptones. These true peptones are formed in relatively large amounts in pancreatic digestion, while in pepsin digestion they are formed only in small quantities or after prolonged digestion.

According to SCHÜTZENBERGER and KÜHNE[3] the proteins yielded two chief groups of new protein bodies when decomposed by dilute mineral acids or with proteolytic enzymes; of these the *anti group* shows a greater resistance to further action of the acid and enzyme than the other namely, the *hemi group*. These two groups are, according to KÜHNE, united in the different proteoses, even though in various relative amounts, and each proteose contains the anti as well as the hemi group. The same is true for the peptone obtained in pepsin digestion, hence he calls it *amphopeptone*. In tryptic digestion a cleavage of the ampho-

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[1] Pflüger's Archiv, **34**.

[2] See Kühne, Verhandl. d. naturhistor. Vereins zu Heidelberg (N. F.), **8**; J. Wenz, Zeitschr. f. Biologie, **22**; Kühne and Chittenden, Zeitschr. f. Biologie, **22**; R. Neumeister, *ibid.*, **23**; Kühne, *ibid.*, **29**.

[3] Schützenberger, Bull. de la Soc. chimique de Paris, **23**; Kühne, Verhandl. d. naturhist. Vereins zu Heidelberg (N. F.), **1**, and Kühne and Chittenden, Zeitschr. f. Biologie, **19**. See also Paal, Ber. d. deutsch. chem. Gesellsch., **27**.

peptone takes place into *antipeptone* and *hemipeptone*. Of these two peptones the hemipeptone is further split into amino-acids and other bodies while the antipeptone is not attacked. By the sufficiently energetic action of trypsin only one peptone remains to the last—the so-called antipeptone.

KÜHNE and his pupils, who have conducted extensive investigations on the proteoses and peptones, classify the various proteoses according to their different solubilities and precipitation properties. In the pepsin digestion of fibrin [1] they obtained the following proteoses: (*a*) *Heteroproteose*, insoluble in water but soluble in dilute salt solution; (*b*) *Protoproteose*, soluble in salt solution and water. These two proteoses are precipitated by NaCl in neutral solutions, but not completely. Heteroproteose may, by being in contact with water for a long time or by drying, be converted into a modification, called (*c*) *Dysproteose*, which is insoluble in dilute salt solutions. (*d*) *Deuteroproteose* is a proteose which is soluble in water and dilute salt solution and which is incompletely precipitated from acid solution by saturating with NaCl, and is not precipitated from neutral solutions.

The proteoses obtained from different protein bodies do not seem to be identical, but differ in their behavior to precipitants. Special names have been given to these various proteoses according to the mother-protein, namely, *albumoses*, *globuloses*, *vitelloses*, *caseoses*, *myosinoses*, *elastoses*, etc. These various proteoses are further distinguished, as *proto-*, *hetero-*, and *deuterocaseose*, for example. CHITTENDEN [2] has suggested the common name proteoses for the products formed intermediary between the proteins and peptones in the digestion of animal and vegetable proteins. We have made use of it in this sense in preference to the word albumose (which is used in the German and by some other writers), but which will be used in this book as indicating the intermediary products in the hydrolysis of albumins and not as a general term. Certain proteoses have also been obtained in a crystalline state (SCHRÖTTER).

NEUMEISTER [3] designates as *atmidalbumose* that body which is obtained by the action of superheated steam on fibrin. At the same time he also obtained a substance called *atmidalbumin*, which stands between the albuminates and the proteoses.

Of the soluble proteoses NEUMEISTER designates the protoproteose and heteroproteose as *primary proteoses*, while the deuteroproteoses, which are closely allied to the peptones, he calls *secondary proteoses*. As essential differences between the primary and secondary proteoses he

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[1] See Kühne and Chittenden, Zeitschr. f. Biologie, **20**.

[2] Kühne and Chittenden, Zeitschr. f. Biologie, **22** and **26**; Neumeister, *ibid.*, **23**; Chittenden and Hartwell, Journ. of Physiol., **11** and **12**; Chittenden and Painter, Studies from the Laboratory, etc., Yale University, **2**, New Haven, 1887; Chittenden, *ibid.*, **3**; Sebelien, Chem. Centralblatt, 1890; Chittenden and Goodwin, Journ. of Physiol., **12**.

[3] Zeitschr. f. Biologie, **26**. See also Chittenden and Meara, Journ. of Physiol., **15**, and Salkowski, Zeitschr. f. Biologie, **34** and **37**.

suggests the following.[1] The primary proteoses are precipitated by nitric acid in salt-free solutions, while the secondary proteoses are precipitated only in salt solutions, and certain deuteroproteoses, such as deuterovitellose and deuteromyosinose, are precipitated by nitric acid only in solutions saturated with NaCl. The primary proteoses are precipitated from neutral solutions by copper-sulphate solution (2:100), and by NaCl in substance, while the secondary proteoses are not. The primary proteoses are completely precipitated from a solution saturated with NaCl by the addition of acetic acid saturated with salt, while the secondary proteoses are only partly precipitated. The primary proteoses are readily precipitated by acetic acid and potassium ferrocyanide, while the secondary are only incompletely precipitated after some time. The primary proteoses are also, according to PICK,[2] completely precipitated by ammonium sulphate (added to one-half saturation), while the secondary proteoses remain in solution.

The true peptones, as they were formerly considered to be, are exceedingly hygroscopic, and if perfectly dry, sizzle like phosphoric anhydride when treated with a little water. They are exceedingly soluble in water, diffuse more readily than the proteoses, and are not precipitated by ammonium sulphate. In contradistinction to the proteoses, the true peptones are not precipitated by nitric acid (even in solutions saturated with salt), by sodium chloride and acetic acid saturated with salt, potassium ferrocyanide and acetic acid, picric acid, trichloracetic acid, potassium-mercuric iodide, and hydrochloric acid. They are precipitated by phosphotungstic acid, phosphomolybdic acid, corrosive sublimate (in the absence of neutral salts), absolute alcohol, and tannic acid, but the precipitate may redissolve on the addition of an excess of the precipitant. As an important difference between amphopeptone and antipeptone we must also mention that the former gives MILLON'S reaction, while the antipeptone does not.

In regard to the precipitation by alcohol we must call attention to the observations of FRÄNKEL that not only are the acid combinations of peptone (PAAL) soluble in alcohol, but also the free peptone, and FRÄNKEL has even suggested a method of preparation based on this behavior. SCHRÖTTER[3] has also prepared crystalline proteoses which were soluble in hot alcohol, especially methyl alcohol.

The views on the hydrolytic cleavage products of peptic and tryptic digestion which were accepted until a few years ago have recently been considerably modified in several points.

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[1] Neumeister, Zeitschr. f. Biologie, **24** and **26**.

[2] Zeitschr. f. physiol. Chem., **24**.

[3] Fränkel, Zur Kenntnis der Zerfallsprodukte des Eiweisses bei peptischer und tryptischer Verdauung, Wien, 1896; Schrötter, Monatshefte f. Chem., **14** and **16**.

The older view that in peptic digestion only proteoses and peptones, but no simpler cleavage products, are formed, has been shown not to be true. The works of ZUNZ, PFAUNDLER, SALASKIN, LAWROW, LANGSTEIN,[1] and others have shown that by very lengthy digestion amino-acids may in part be formed and also other products such as oxyphenylethylamine, tetra- and pentamethylenediamine. The biuret reaction does not disappear and the above mentioned products seem to be formed only under special conditions. In ordinary, not too lengthy pepsin, digestion, it is generally admitted that no amino acids are formed but only proteoses and peptones.

In connection with the above-mentioned experimental results it must be remarked that not all the products found, for example, the oxyphenylethylamine and the diamines, are produced by the action of pepsin, but rather by the action of other enzymes. In certain cases, undoubtedly, impure pepsin was used, or indeed autodigestion of the stomach was carried on, and the action of other enzymes was not excluded. In other cases the digestion with pepsin and considerable acid (even 1 per cent H_2SO_4) was continued for a very long time, indeed for an entire year, without controlling the influence of the acid alone upon the proteoses.

KÜHNE'S view that in tryptic digestion (pancreatic digestion) a peptone, so-called antipeptone, always remains which cannot be further split is not strictly true. By sufficiently long autodigestion of the pancreas, KUTSCHER[2] was able to obtain, as final products, a mixture of digestion products which failed to respond to the biuret test, and the same results have been obtained by others. An antipeptone in the old sense, i.e., a digestion product which is resistant to tryptic digestion but which still gives the biuret test, is without question not always obtained as end product in trypsin digestion. On the contrary as FISCHER and ABDERHALDEN[3] have shown, polypeptide-like bodies are produced in tryptic digestion (and the same is probably true also for peptic digestion) which do not give the biuret test, i.e., "abiuret" products, and which are resistant to further tryptic digestion but yield amino-acids on hydrolysis with acids. This behavior stands in close relation to the observation that in tryptic digestion certain amino-acids, such for example, as tyrosine, tryptophane and leucine are split off earlier and more readily than the others of the protein molecule.

Antipeptone, which is only attacked with great difficulty by trypsin has in fact been isolated by SIEGFRIED (see below) and although the

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[1] Zunz, Zeitschr. f. physiol. Chem., **28**, and Hofmeister's Beiträge, **2**; Pfaundler, Zeitschr. f. physiol. Chem., **30**; Salaskin, *ibid.*, **32**; Salaskin and Kowalewsky, *ibid.*, **38**; Lawrow, *ibid.*, **33**; Langstein, Hofmeister's Beiträge, **1** and **2**.

[2] Zeitschr. f. physiol. Chem., **25**, **26**, **28**, and Die Endprodukte der Trypsinverdauung, Habilitationsschrift Strassburg, 1899.

[3] Zeitschr. f. physiol. Chem., **39**.

views of KÜHNE are not in all points correct still the fact remains that under certain circumstances the protein can be split into fractions, of which the hemi group is further easily decomposed by enzyme action while the other, the anti group, is very much more resistant to such action. It also seems as if the first group is characterized by a larger content of tyrosine, tryptophane and the latter by its content of glycocoll, phenylalanine and proline.

By the use of the methods specially worked out by the HOFMEISTER school, of fractionally salting out with ammonium sulphate or zinc sulphate or also by SIEGFRIED'S iron-alum method, numerous attempts to separate the various proteoses and peptones have been made.[1] Not only have we learned by these methods of a larger number of proteoses, but our older conception of the products formed primarily has been materially modified. Immediately at the commencement of digestion, even in peptic digestion, a splitting of the protein molecule into several complexes takes place. In opposition to the view of HUPPERT,[2] that the proteoses, in pepsin digestion, are always derived from the primarily formed acid albuminate, PICK and ZUNZ have shown that several proteoses, as well as acid albuminate, appear as primary products at the commencement of the digestion. According to GOLDSCHMIDT[3] a splitting off of proteoses and the formation of acid albuminate takes place simultaneously by the action of dilute acids alone. Besides the proteoses we also have, according to ZUNZ and PFAUNDLER, even at the beginning, other primary bodies, which cannot be salted out and which do not give the biuret reaction, but are in part precipitated by phosphotungstic acid. These little-known products seem to be intermediate between the peptones and the amino-acids, and they correspond probably to the polypeptide bodies obtained by FISCHER and ABDERHALDEN in tryptic digestion.

By fractional precipitation of WITTE'S peptone with ammonium sulphate PICK has obtained various chief fractions of proteoses. The first contains the proto- and heteroproteoses whose precipitation limit lies at 24–42 per cent saturation with ammonium sulphate solution, i.e., the presence of 24–42 cc. of the saturated ammonium sulphate solution in 100 cc. of the liquid. Then follows a fraction A at 54–62 per cent saturation, then a third fraction B, with 70–95 per cent saturation, and finally fraction C, which precipitates from the saturated solution on acidification with sulphuric acid saturated with the salt.

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[1] Umber, Zeitschr. f. physiol. Chem., **25**; Alexander, *ibid.*, **25**; Pfaundler, *ibid.*, **30**; Zunz, *ibid.*, **28**, and Hofmeister's Beiträge, **2**; Pick, *ibid.*, **2**, and Zeitschr. f. physiol. Chem., **24** and **28**; Siegfried, see footnote 3, p. 136.

[2] Schütz and Huppert, Pflüger's Arch., **80**.

[3] F. Goldschmidt, Ueber die Einwirkung von Säuren auf Eiweisstoffe, Inaug.-Diss. Strassburg, 1898.

The hetero- and protoproteoses are not, according to our present views, the only primary proteoses. In the proteose fraction obtained on saturating with ammonium sulphate in neutral liquids, which should contain secondary proteoses only, primary proteoses such as the *glucoproteose* (PICK), which contains a carbohydrate group and the so-called *synproteose* (HOFMEISTER [1]) occur. It is no longer sufficient to consider an unequal ability to be salted out, as an essential difference between the primary and secondary proteoses.

There is no doubt that there exists a large number of so-called proteoses having various precipitation properties, and different other properties and new differences appear, while working with them according to different methods. For example RONA and MICHAELIS [2] find that certain proteoses are precipitated by mastic emulsion while others are not. Those that are precipitatable by mastic, can all be salted out, while all those that can be salted out are not all precipitated by mastic. The hetero- and protoproteoses act, according to ZUNZ [3] like strong protection colloids toward colloidal gold, which is not the case with the others, and also, according to this worker, the so-called proteoses are more readily precipitated by chondroitin-sulphuric acid and acetic acid than the so-called secondary proteoses. According to HUNTER [4] only the primary proteoses are precipitated by protamines while the secondary are not. It is also possible that numerous intermediary members exist between those proteoses which stand close to the original protein and those that are further removed. The difficulties in isolation and purification of these different members are so very great that the proteoses thus far isolated must not be considered as chemical individuals. Under these circumstances the above-mentioned differentiation and classification of the various proteoses is of little value and a more detailed discussion of the properties of the various proteoses thus far isolated is without interest.

It would be of great interest if certain differences in the chemical structure of the different proteoses could be determined with certainty. Such differences are claimed to have been found in certain cases. Thus HART has found that the heteroproteose (from muscle syntonin) was considerably richer in arginine and poorer in histidine than the protoproteose, and PICK has also found marked differences between the hetero- and protoproteose from fibrin. The hetero-proteose yields very little

[1] Ueber Bau und Gruppirung der Eiweisskörper, Ergebnisse der Physiol., Jahrg. I, Abt. 1, 783.

[2] Biochem. Zeitschr., 8.

[3] Arch. internat. d. Physiol., 1 and 5, and Bull. Soc. Scienc. méd. et natur. Bruxelles, 64.

[4] Journ. of Physiol., 37.

tyrosine and indol but abundant leucine and glycocoll, and about 39 per cent of the total nitrogen in a basic form. The protoproteose, according to PICK, on the contrary yields considerable tyrosine and indol, only little leucine but no glycocoll, and contains only about 25 per cent basic nitrogen. FRIEDMANN, HART, and LEVENE have obtained very similar results in regard to the quantity of basic nitrogen in the two-proteoses, although LEVENE as well as ADLER [1] did not find the same results as PICK in regard to the amounts of monamino-acids in the two proteoses. The work of LEVENE, v. SLYKE and BIRCHARD [2] show, in many important points, a decided contradiction to the statements of PICK and these divergent results may possibly be explained by the fact that they were not working with pure substances, but rather with mixtures.

According to PICK the heteroproteose is also more resistant toward tryptic digestion than the protoproteose, a behavior which coincides with KÜHNE's view of a resistant atomic complex, an anti group, in the protein bodies. KÜHNE and CHITTENDEN [3] regularly obtained on the tryptic digestion of heteroproteose a separation of so-called antialbumid, a body which is attacked with great difficulty in tryptic digestion, but which separates as a jelly-like mass and which is richer in carbon (57.5–58.09 per cent), but poorer in nitrogen (12.61–13.94 per cent), than the original protein. The occurrence of such resistant complexes in digestion has also been repeatedly observed.

This antialbumid later attracted increased interest, because as first found by DANILEWSKY and later other investigators have shown, that solutions of rennin, gastric juice, pancreatic juice, and papain cause a similar coagulum in not too dilute proteose solutions. These coagula, called *plasteines* (coagulum by rennin) by SAWJALOW, and *coaguloses* (coagulum by papain) by KURAJEFF,[4] are similar in many respects to antialbumid, having a higher content of carbon (57–60 per cent) and nitrogen (13–14.6 per cent). In other cases the quantity of carbon as well as nitrogen is lower (LAWROW).

We cannot for the present make any positive statement as to the importance and mode of formation of the coaguloses or plasteins. It

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[1] Hart, Zeitschr. f. physiol. Chem., **33**; Pick, *ibid.*, 28; Friedmann, *ibid.*, **29**; Levene, Journ. of Biol. Chem., 1; R. Adler, Die Heteroalbumose und Protalbumose des Fibrins Dissert. Leipzig, 1907.

[2] Journ. of Biol. Chem., 8 and 10.

[3] Zeitschr. f. Biol., 19 and 20.

[4] The works of Danilewsky and Okunew are cited and reviewed in the following Sawjalow, Pflüger's Arch., 85, and Centralbl. f. Physiol., 16; and Zeitschr. f. physiol. Chem., 54; Lawrow and Salaskin, Zeitschr. f. physiol. Chem., 36; Lawrow, *ibid.*, 51, 53, 56 and 60; Kurajeff, Hofmeister's Beiträge, 1 and 2; see also Sacharow, Biochem. Centralbl., 1, 233; Levene and v. Slyke, Biochem. Zeitschr., 13.

is rather generally admitted that they are formed by a synthesis, a view which has received support by the investigations of V. HENRIQUES and GJALDBÄK.[1] According to SAWJALOW a plastein is not formed from a proteose alone, but always from a mixture of proteoses. LAWROW claims that they may be produced from proteoses as well as from polypeptide substances, and correspondingly we must differentiate between the coaguloses or coagulosogens from the proteose group *coaproteoses*, and from the polypeptide group or *coapeptides*. The latter yield on hydrolysis chiefly monamino-acids, while the first yield also basic nitrogenous products. Perhaps the plasteinogen investigated by BAYER[2] which differs essentially from the true proteid in its elementary composition as well as from other coaguloses, belongs to the coapeptides.

The different behavior on saturating their solution with ammonium sulphate has been generally used, as above remarked, for years to differentiate between the proteoses and peptones. Those precipitable by this salt were called proteoses, and those not were called peptones. This method of division, which never had sufficient support and which was perfectly arbitrary, cannot be considered at the present time. We know now, thanks to the works of EMIL FISCHER and his co-workers, that there are polypeptides either prepared artificially or found among the cleavage products of the proteins, which are precipitated by ammonium sulphate. At the present it is generally conceded that the peptones in the ordinary sense are only a mixture of different bodies. The chief step in these investigations must be the isolation from this mixture of unit bodies with definite chemical characteristics. Of such bodies, besides the polypeptides previously mentioned and studied by FISCHER and others, we must mention the products isolated by SIEGFRIED and his pupils.[3]

These so-called peptones are in part peptic-peptones and partly tryptic-peptones, and some are prepared from proteid (fibrin) and others from gelatin. The tryptic fibrin-peptones are antipeptones in KÜHNE'S sense because they are very resistant to the further action of trypsin. They are according to NEUMANN simultaneously bibasic acids and monoacidic bases. They give the biuret reaction, but not MILLON'S reaction; they contain no tyrosine and yield on hydrolysis, arginine, lysine, glutamic acid, and it seems also aspartic acid. A pepsin-glutin peptone isolated by SIEGFRIED and SCHMITZ yielded arginine, lysine, glutamic acid, gly-

[1] Zeitschr. f. physiol. Chem., **71** and **81**.

[2] Hofmeister's Beiträge, **4**; see also L. Rosenfeld, *ibid.*, **9**; J. Lukomnik, *ibid.*, **9** and F. Micheli, Biochem. Centralbl., **6**, p. 562.

[3] The works of Siegfried and his pupils, Fr. Müller, Borkel, Mühle, Krüger, Scheermesser, Neumann, H. Schmitz, may be found in Arch. f. (Anat. u.) Physiol., 1894 and Zeitschr. f. physiol. Chem., **21**, **41**, **43**, **45**, **48**, **50**, and **65** and Pflüger's Arch. 136.

cocoll and besides these also leucine and proline although not in quantities that could be determined. Of the total nitrogen they found 19.7 per cent arginine, 9.1 per cent lysine, 49.2 per cent glycocoll, 9.3 percent glutamic acid and 12.7 per cent proline and leucine together. SIEGFRIED has given proof in several ways as to the purity and unity of the peptones isolated by him.

In another manner, namely by fractional precipitation with metallic salts, especially with mercuric-potassium iodide and the preparation of phenylisocyanate compounds, HOFMEISTER and his pupils STOOKEY, RAPER and ROGOZINSKI [1] have isolated peptones or polypeptide-like bodies from blood proteid. One of these, called arginine-histidine peptone, yielded arginine and histidine as basic hydrolytic products while another yielded chiefly lysine as basic product and hence was called lysine-peptone.

From glutin-peptone, SIEGFRIED, on warming with hydrochloric acid, obtained a base, $C_{21}H_{39}N_9O_8$, which can also be directly obtained from gelatin. This he calls a *kyrin*, because it is to be considered as a basic protein nucleus, and he calls this special one *glutokyrin*. The glutokyrin gives the biuret reaction and is considered as a basic peptone. On complete hydrolytic cleavage it yields arginine, lysine, glutamic acid, and glycocoll. Of the total nitrogen two-thirds belong to the bases and one-third to the amino-acids. Recently he with O. PILZ on further hydrolysis has prepared a β-glutokyrin, which only yielded arginine, lysine and glutamic acid. Similar basic nuclei, *protokyrins*, have recently been obtained by SIEGFRIED [2] from fibrin and casein, using the same method. Caseinokyrin gives a non-crystalline sulphate, but a crystalline phosphotungstate. The free caseinokyrin has an alkaline reaction, gives the biuret test, and its composition corresponds to the formula $C_{23}H_{47}N_9O_8$. It yields arginine, lysine, and glutamic acid on cleavage. The basic nitrogen amounts to about 85 per cent of the total nitrogen, and caseinokyrin, behaves in this respect like a protamine.

Among the known cleavage products of proteins, arginine is the only one which, up to the present, is never absent, and for this reason we designate as proteins only those atomic complexes which contain, besides chained monamino-acids, also arginine, or, more simply, show the previously mentioned imide bindings. Hence caseinokyrine, which yields only arginine, lysine and glutamic acid, and scombrin, which yields only arginine, proline, and alanine, are the simplest known proteins.

Scombrin belongs to the previously mentioned group of protamines which, according to KOSSEL,[3] are formed by a successive cleavage of the

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[1] Hofmeister's Beiträge, 7, 9, and 11.

[2] Kgl. Sächs. Ges. d. Wiss., Math.-Phys. Klasse, 1903, and Zeitschr. f. physiol Chem., 43, with Pilz., *ibid.*, 58.

[3] Zeitschr. f. physiol. Chem., 44.

typical protein. The occurrence of basic protokyrins in the hydrolytic cleavage of genuine proteins like gelatin has given valuable support to KOSSEL's theory as to a basic nucleus in the protein bodies.

On account of the cleavage taking place in digestion, the digestive products should have a lower molecular weight than the original protein. This is really the case as shown by molecular weight determinations. As these determinations have been made upon impure substances or mixtures, the results[1] obtained are only of little value. The same is true for the elementary analysis of the proteoses and peptones.[2]

In the preparation and separation of various proteoses and peptones all precipitable protein is always removed first by neutralization and then by boiling. The proteoses may then be separated from the peptones by means of ammonium sulphate according to KÜHNE's method, and divided into different fractions according to the method of PICK and the HOFMEISTER school. The separation and preparation of pure hetero- and protoproteoses can be best performed by the method suggested by PICK, but this method, as well as that with ammonium sulphate, gives good results only when the precautions suggested by HASLAM[3] are carefully followed. We can here only refer to the cited works of KÜHNE and co-workers, of E. ZUNZ and especially those of the HOFMEISTER and the SIEGFRIED schools. In regard to the literature on the detection of proteoses and peptones in animal fluids we refer to Chapters V and XIV.

If we wish to detect the presence of so-called true peptone, by means of the biuret reaction in a solution saturated with ammonium sulphate, we add a slight excess of a concentrated solution of caustic soda and cool, and then add a two per cent solution of copper sulphate drop by drop, after the sodium sulphate has separated out.

In the quantitative estimation of proteoses and peptones we make use of the nitrogen estimation, the biuret test (colorimetric), and the polarization method. These methods do not give exact results.

The polypeptides have had their most important properties discussed on pages 85–91, and of the cleavage products of the proteins only the amino-acids remain to be discussed.

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[1] Sabanejew, Ber. d. d. chem. Gesellsch., **26**, 385; Paal, *ibid.*, **27**, 1827; Sjöqvist, Skand. Arch. f. Physiol., **5**.

[2] Elementary analyses of proteoses and peptones will be found in the works of Kühne and Chittenden and their pupils, cited in footnote 2, p. 130; also by Herth, Zeitschr. f. physiol. Chem., **1**, and Monatshefte f. Chem., **5**; Maly, Pflüger's Arch. **9**, **20**; Henninger, Compt. rend., **86**; Schrötter, l. c., Paal, l. c.

[3] Journ. of Physiol., **32** and **36**.

3. The Amino-acids.[1]

Glycocoll (amino-acetic acid), $C_2H_5NO_2 = \begin{matrix} CH_2(NH_2) \\ COOH \end{matrix}$, also called glycine or gelatin sugar, is found in the muscles of the invertebrates, but has chief interest as a hydrolytic decomposition product of protein bodies, especially fibroin, spider-silk elastin, gelatin, and spongin, as well as of hippuric acid and glycocholic acid.

Glycocoll forms colorless, often large, hard rhombic crystals or four-sided prisms. The crystals have a sweet taste and dissolve readily in cold water (4.3 parts). Glycocoll is insoluble in alcohol and ether and dissolves with difficulty in warm alcohol. Like the amino-acids in general it combines with acids and alkalies. With the latter compounds we must mention those with copper and silver. Glycocoll dissolves cupric hydroxide in alkaline liquids, but does not reduce at boiling heat. A boiling-hot solution of glycocoll dissolves freshly precipitated cupric hydroxide, forming a blue solution, which in proper concentration deposits blue needles of copper-glycocoll on cooling. The compound with hydrochloric acid is readily soluble in water but less soluble in alcohol.

SÖRENSEN[2] finds that phosphotungstic acid does not precipitate glycocoll from dilute solutions but only from concentrated ones. By the action of gaseous HCl upon glycocoll in absolute alcohol, beautiful crystals are obtained of the hydrochloride of glycocoll-ethyl ester, which melts at 144° C. and from which the glycocoll-ethyl ester can be obtained by the method suggested by E. FISCHER[3] for the separation of glycocoll from the other amino-acids. On shaking with benzoyl chloride and caustic soda, hippuric acid is formed, and this is also made use of in different ways in detecting and isolating glycocoll (CH. FISCHER, GONNERMANN, SPIRO[4]). The β-naphthalene-sulpho-glycine with a melting-point of 159°, the 4-nitro-tolulene-2-sulpho-glycine, melting at 180°, and the α-naphthylisocyanate compound melting at 190.5–191.5° are also of importance. On putrefaction methane is probably produced from glycocoll.

Glycocoll can be best prepared from hippuric acid by boiling it with 4 parts dilute sulphuric acid (1 : 6) for ten to twelve hours. After cooling

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[1] In regard to the division of the amino-acids among the three chief groups of organic compounds we refer to pages 85–86.

[2] Meddelelser, fraa Carlsberg-laboratoriet, **6**, 1905.

[3] Ber. d. d. chem. Gesellsch., **34**.

[4] Ch. Fischer, Zeitschr. f. physiol. Chem., **19**; Spiro, *ibid.*, **28**; Gonnermann, Pflüger's Arch., **59**.

the benzoic acid is removed, the filtrate concentrated, the remaining benzoic acid removed by extracting with ether, the sulphuric acid precipitated by $BaCO_3$, and the filtrate evaporated to the point of crystallization. (In regard to its preparation from protein substances see below.)

d-Alanine (α-aminopropionic acid), $C_3H_7NO_2 = \begin{matrix} CH_3 \\ | \\ CH(NH_2) \\ | \\ COOH \end{matrix}$. The *d*-alanine is obtained in relatively small amounts from the true proteids, but in larger quantities from the albuminoids, especially from fibroin, spider-silk and elastin.

d-alanine has been prepared from *l*-serine by E. FISCHER and K. RASKE,[1] and FISCHER has also obtained it from racemic alanine by splitting the benzoyl combination, or from *l*-alanine by splitting with yeast by WALDEN'S reversion.

Alanine generally crystallizes in needles or oblique rhombic columns. It is very readily soluble in water, having a sweetish taste, and dissolves cupric hydroxide on boiling, producing a deep blue solution of a crystallizable copper salt. Alanine is insoluble in absolute alcohol. The rotation of alanine at 20° C. in aqueous solution is $(a)_D = +2.7°$ and for a solution in hydrochloric acid (9–10 per cent solution) is $(a)_D = +10.3°$.

The β-naphthalene-sulpho-*d*-alanine melts, when dry, at about 123° and sinters at 117° C. The phenylisocyanate melts at 168° and the α-naphthylisocyanate alanine melts at 198°. On putrefaction alanine yields propionic acid.

d-Valine (α-amino-valeric acid), $C_5H_{11}NO_2 = \begin{matrix} CH_3 & CH_3 \\ & \diagdown\diagup \\ & CH \\ & | \\ & CH(NH_2) \\ & | \\ & COOH \end{matrix}$, has been detected several times among the cleavage products of protein substances, although only in small quantities. KOSSEL and DAKIN obtained 4.3 per cent valine from salmine, and E. FISCHER and DÖRPINGHAUS[2] 5.7 per cent from horn substance. The largest quantity has been obtained from casein and edestin, namely, 7.20 and 5.6 per cent respectively. Because of the difficulty in separating valine from the two leucines[3] the figures given are somewhat uncertain. The acid isolated by H. and E. SALKOWSKI[4] from putrefying proteid or gelatin seems to have been δ-amino-*n*-valeric acid.

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[1] Ber. d. d. chem. Gesellsch., **40**.

[2] Kossel and Dakin, Zeitschr. f. physiol. Chem., **41**; Fischer and Dörpinghaus, *ibid.*, **36**.

[3] See Levene and v. Slyke, Journ. of Biol. Chem., **6**.

[4] Ber. d. d. chem. Gesellsch., **16** and **31**.

d-valine can be obtained as microscopic crystalline leaves. It is rather readily soluble in water and the solution has a faint sweetish taste and at the same time somewhat bitter. The solution has a rotation of $(a)_D = +6.42°$. The hydrochloric acid solution (20 per cent) shows, according to FISCHER, a rotation of $(a)_D = +28.8°$. The copper salt, which forms leaves which are rather soluble in water, is very easily soluble in methyl alcohol (SCHULZ and WINTERSTEIN[1]).

The phenylisocyanate melts at 147°, and on boiling with 20 per cent hydrochloric acid for a short time, it is changed into *d*-phenylisopropyl hydantoin, which melts at 131–133° C.

On putrefaction valine yields isobutylamine and isovaleric acid.

***l*-Leucine** (aminocaproic acid, or, more correctly, α-aminoisobutylacetic acid), $C_6H_{13}NO_2 = \begin{matrix} CH_3CH_3 \\ \diagdown\diagup \\ CH \\ | \\ CH_2 \\ | \\ CH(NH_2) \\ | \\ COOH \end{matrix}$, is produced from protein substances in their hydrolytic cleavage by proteolytic enzymes, by boiling with dilute acids or alkalies or by fusing with alkali hydroxides, and by putrefaction. There are also observations that indicate that in the hydrolysis besides the ordinary leucine perhaps also normal leucine may be formed (HECKEL and SAMEC[2]).

Because of the ease with which leucine (and tyrosine) are formed in the decomposition of protein substances, it is difficult to decide positively whether these bodies when found in the tissues are constituents of the living body or are to be considered only as decomposition products formed after death. Leucine, it seems, has been found as a normal constituent of the pancreas and its secretion, in the spleen, thymus, and lymph glands, in the thyroid gland, in the salivary glands, in the kidneys and in the liver. It also occurs in the wool of sheep, in dirt from the skin (inactive epidermis), and between the toes, and its decomposition products have the disagreeable odor of the perspiration of the feet. It is found pathologically in atheromatous cysts, ichthyosis scales, pus, blood, liver, and urine (in diseases of the liver and in phosphorus poisoning). Leucine often occurs in invertebrates and also in the plant kingdom. On hydrolytic cleavage various protein substances yield different amounts of leucine, as shown in the tables given on pages 106, 107, 115 and 125. From the figures, there given, we call attention to the following: ERLENMEYER and SCHÖFFER obtained 36–45 per cent leucine from the cervical ligament, E. FISCHER and ABDERHALDEN 20 per cent from hæmo-

[1] Zeitschr. f. physiol. Chem., **35**.

[2] Heckel, Monatsh. f. Chem., **29**; Samec, *ibid.*, **29**.

globin, and FISCHER and DÖRPINGHAUS 18.3 per cent from horn substance.[1]

The leucine obtained by cleavage of protein substances is generally *l*-leucine, which is levorotatory in water solution and dextrorotatory in acid solution. The leucine prepared synthetically by HÜFNER[2] from isovaleraldehyde, ammonia, and hydrocyanic acid is optically inactive. Inactive leucine may also be prepared, by the cleavage of proteins with baryta at 160–180° C., because of a ready racemation. The *d-l*-leucine may be split into the two components by various means, especially by the preparation of the formyl combination.[3]

On oxidation the leucines yield the corresponding oxyacids (leucinic acids). Leucine is decomposed on heating, evolving carbon dioxide, ammonia, and amylamine. On heating with alkalies, as also in putrefaction, it yields valeric acid and ammonia. On putrefaction it yields isoamylamine and isocaproic acid.

Leucine crystallizes when pure in shining, white, very thin plates, usually forming round knobs or balls, either appearing like hyaline, or with alternating light and dark concentric layers which consist of radial groups of crystals. By slow heating, leucine melts and sublimes into white woolly flakes, which are similar to sublimed zinc oxide. At the same time an odor of amylamine is developed. Quickly heated in a closed capillary tube, it melts with decomposition at 293–295°.

Leucine, as obtained from animal fluids and tissues is always impure, and is very easily soluble in water and rather easily in alcohol. Pure leucine is soluble with difficulty. Pure *l*- and *d*-leucine dissolve in 40–46 parts water, more readily in hot alcohol, but with difficulty in cold alcohol. The *d-l*-leucine is much less soluble. According to HABERMANN and EHRENFELD[4] 100 parts of boiling glacial acetic acid dissolve 29.23 parts of leucine. The specific rotation of *l*-leucine, dissolved in hydrochloric acid (20 per cent solution) is $(\alpha)_D = +15.6°$ according to FISCHER and WARBURG. In aqueous solution it is $(\alpha)_D = -10.40°$, according to F. EHRLICH and WENDEL.[5]

The solution of leucine in water is not, as a rule, precipitated by metallic salts. The boiling-hot solution may, however, be precipitated by a boiling-hot solution of copper acetate, and this fact is made use of in separating leucine from other substances. If the solution of leucine

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[1] Erlenmeyer and Schöffer, cited from Maly, Chem. d. Verdauungssäfte, in Hermann's Handb. d. Physiol., 5, Theil 2, p. 209; Fischer and his collaborators, *ibid.*, 86.

[2] Journ. f. prakt. Chem. (N. F.), 1.

[3] Fischer and Warburg, Ber. d. d. chem. Gesellsch., 38.

[4] Zeitschr. f. physiol. Chem., 37.

[5] Fischer and Warburg, Ber. d. d. chem. Gesellsch., 38; E. Ehrlich and Wendel, Biochem. Zeitschr., 8.

is boiled with sugar of lead and then ammonia be added to the cooled solution, shining crystalline leaves of leucine-lead oxide separate. Leucine dissolves cupric hydroxide, but does not reduce on boiling.

Leucine is readily soluble in alkalies and acids. It gives crystalline compounds with mineral acids. If leucine hydrochloride is boiled with alcohol containing 3–4 per cent HCl, long narrow crystalline prisms of leucine-ethyl-ester hydrochloride, melting at 134° C., are formed. The picrate of the leucine ester melts at 128°. The phenylisocyanate of *d-l*-leucine melts at 165° and its anhydride at 125° C. The α-naphthyl-isocyanate leucine melts at 163.5°, the naphthalene-sulpho-*l*-leucine at 68° C.

Leucine is recognized under the microscope by the appearance of balls or knobs, by its action when heated (sublimation test), and by its compounds, especially the hydrochloride and picrate of the ethyl ester and the phenylisocyanate compound of the racemic leucine obtained on heating with baryta water, the α-naphthylisocyanate compound and the β-naphthalene-sulpho-leucine. According to the method suggested by LIPPICH [1] the leucine can be transformed into isobutylhydantoin, having a melting-point of 205°, by boiling with an excess of urea and baryta water. For the preparation and separation of leucine from the other amino-acids of the leucine fraction special methods have been suggested by F. EHRLICH and WENDEL, LEVENE and v. SLYKE.[2]

Leucinimide, $C_{12}H_{22}N_2O_2 = \begin{matrix} C_4H_9.CH.NH.CO \\ CO.NH.CH.C_4H_9 \end{matrix}$, was first obtained by RITTHAUSEN in the hydrolytic cleavage products on boiling proteins with acids, and subsequently by R. COHN. SALASKIN [3] obtained it in the peptic and tryptic digestion of hæmoglobin. As an anhydride of leucine (2.5-diacipiperazine) it is probably formed by a secondary change, from leucine.

It crystallizes in long needles and sublimes readily. The melting-point has not been found constant in the different cases. The leucinimide (3.6-diisobutyl-2.5-diacipiperazine) prepared synthetically by E. FISCHER [4] from leucine-ethyl ester melted at 271° C.

***l*-Isoleucine** (β-methyl-ethyl-α-amino-propionic acid),

$$C_6H_{13}NO_2 = \begin{matrix} CH_3C_2H_5 \\ \diagdown\diagup \\ CH \\ | \\ CHNH_2 \\ | \\ COOH \end{matrix}$$

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[1] Ber. d. d. chem. Gesellsch., **39**.

[2] F. Ehrlich and Wendel, l. c.; Levene and v. Slyke, Journ. of Biol. Chem., **6**.

[3] Ritthausen, Die Eiweisskörper der Getreidearten, etc., Bonn, 1872; R. Cohn, Zeitschr. f. physiol. Chem., **22** and **29**; Salaskin, *ibid.*, **32**.

[4] Ber. d. d. chem. Gesellsch., **34**.

is an isomer of leucine discovered by F. EHRLICH,[1] who first isolated it from the mother-liquor after removing the sugar from beet-sugar molasses. He also found it in the hydrolysis of several proteins, and recently it has been found by others among the products of hydrolysis of the proteins. The largest amount thus far found was 2.6 per cent by LEVENE, V. SLYKE and BIRCHARD[2] in a heteroproteose. It seems to be associated regularly with ordinary leucine, forming mixed crystals, which give an impression of a chemical combination and which are difficult to separate. On this account the earlier claims as to the quantity of leucine are somewhat uncertain, as they always refer to leucine containing isoleucine.

The constitution of isoleucine has been explained by EHRLICH through its relation to *d*-amyl alcohol. Just as according to F. EHRLICH valine yields the isobutyl alcohol in alcoholic fermentation so isoleucine yields *d*-amyl alcohol in the fermentation of sugar with yeast. On the other hand, it can also be obtained, in a manner analogous to the synthesis of leucine, from *d*-amyl alcohol (as a mixture of isoleucine and alloisoleucine, the latter is levogyrate and has a different stereometric configuration from the isoleucine). The synthesis of isoleucine has been accomplished in other ways by several investigators.[3] On putrefaction *d*-caproic acid and *d*-valeric acid have been obtained from isoleucine.[4]

Isoleucine crystallizes in leaves or rods and plates of the rhombic form. It is more soluble in water than leucine (1:25.8). Its solutions have a bitter taste and are astringent. It is dextro-rotatory in aqueous as well as in acid solution. In aqueous solution it has a specific rotation of $(\alpha)_D = +9.74°$ and in 20 per cent hydrochloric acid $(\alpha)_D = +36.8°$. Like valine its copper salt is readily soluble in methyl alcohol. The benzoyl combination melts at 116–117°, the benzene sulphoisoleucine at 149–150°, the phenylisocyanate combination at 119–120°, and the naphthylisocyanate combination at 178° C.

In the leucine fraction, from the amino-acids contained in nerve substance, ABDERHALDEN and WEIL[5] have obtained a new amino-acid, $C_6H_{13}NO_2$ which is isomeric with leucine and which seems to be *d*-α-amino-*n*-caproic acid and called *d-caprine* by them. When crystallized from water it forms six-sided plates which unite to tufts having a faint sweet taste. At 280° (uncorrected) it softens and at 285° (uncorrected)

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[1] Felix Ehrlich, Ber. d. d. chem. Gesellsch., **37**.

[2] Journ. of Biol. Chem., 8.

[3] Ehrlich, Ber. d. d. chem. Gesellsch., **40** and **41**; Brasch and Friedmann, Hofmeister's Beiträge, **11**; Bouveault and Locquin, Compt. rend., **141**, and Bull. soc. chim. (3), **35**; Locquin, Bull. soc. chim. (4), **1**.

[4] C. Neuberg, Bioch. Zeitschr., **37**.

[5] Zeitschr. f. physiol. Chem., **81** and **84**.

it sublimes. Its solubility in water is 1.5:100; at 20° in aqueous solution $(\alpha)_D+6.53°$ and in 20 per cent hydrochloric acid $+14.1°$. It gives a copper salt crystallizing in needles.

l-**Serine** (α-amino-β-oxypropionic acid), $C_3H_7NO_3=\begin{matrix}CH_2(OH)\\CH(NH_2)\\COOH\end{matrix}$, was obtained by FISCHER and his collaborators as a cleavage product of several proteins, generally only in small quantities. The largest quantity, 6.6 per cent, was obtained by FISCHER and SKITA from sericine; KOSSEL and DAKIN [1] obtained a still larger amount from salmine, namely 7.8 per cent. The racemic serine is the one generally obtained. From fibroin FISCHER [2] obtained a mixture of active and inactive serine anhydride from which he finally prepared *l*-serine by hydrolysis. Serine has also been found by G. EMBDEN and TACHAU [3] in fresh perspiration.

Synthetically *d-l*-serine has been prepared by FISCHER and LEUCHS from ammonia, hydrocyanic acid and glycol aldehyde, and also in other ways by others.[4] FISCHER and JACOBS [5] have prepared *l*-serine from *d-l*-serine by the preparation of the alkaloid salt of the *p*-nitro-benzoyl combination. On reduction serine is transformed into alanine, and on oxidation with nitrous acid it yields glyceric acid. The relation of serine to alanine, lactic acid and glyceric acid is evident from the following formulæ:

$CH_2(OH)$	CH_3	CH_3	$CH_2(OH)$
$CH(NH_2)$	$CH(NH_2)$	$CH(OH)$	$CH(OH)$
$COOH$	$COOH$	$COOH$	$COOH$
Serine	Alanine	Lactic acid	Glyceric acid

The *l*-serine crystallizes in thin leaves or crusts. It is rather readily soluble in water; the *d-l*-serine is soluble in 23 parts water at 20° C. The solution of *l*-serine has a sweet taste with an insipid after taste. The specific rotation in aqueous solution at 20° C. is $(\alpha)_D=-6.83°$ and the hydrochloric acid solution at 25° C. is $(\alpha)_D=+14.45°$. The β-napthalene-sulpho-serine melts at 220° C. when anhydrous. The *l*-serine anhydride, which is identical with that obtained from fibroin, forms thin, colorless needles which melt at 247° with decomposition. Its specific rotation in aqueous solution at 25° C. $(\alpha)_D=-67.46°$.

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[1] Fischer and Skita, Zeitschr. f. physiol. Chem., **35**; Kossel and Dakin, *ibid.*, **41**.

[2] Ber. d. d. chem. Gesellsch., **40**.

[3] Bioch. Zeitschr., **28**.

[4] Fischer and Leuchs, Ber. d. d. chem. Gesellsch, **35**; Erlenmeyer and Stoop, *ibid.*, **35**; Leuchs and Geiger, *ibid.*, **39**.

[5] Ber. d. d. chem. Gesellsch., **39**.

Isoserine (β-amino-α-oxypropionic acid) has been prepared by ELLINGER from diamino-propionic hydrobromide and silver nitrite, and by NEUBERG and SILBERMANN from the hydrochloric acid combination of diamino-propionic acid. Other syntheses have been made by NEUBERG and MAYER and by NEUBERG and ASCHER.[1]

***l*-Aspartic acid** (aminosuccinic acid), $C_4H_7NO_4 = \begin{matrix}\dot{C}OOH\\ \dot{C}H(NH_2)\\ \dot{C}H_2\\ \dot{C}OOH\end{matrix}$, has been obtained on the cleavage of protein substances by proteolytic enzymes as well as by boiling them with dilute mineral acids in comparatively small quantities. This acid also occurs in secretions of sea-snails (HENZE[2]) and is very widely diffused in the vegetable kingdom as the amide Asparagine (aminosuccinic-acid amide), which seems to be of the greatest importance in the development and formation of the proteins in plants. *d-l*-Aspartic acid has been prepared synthetically from fumaric acid and alcoholic ammonia. On putrefaction of aspartic acid, propionic acid and succinic acid are formed.

l-Aspartic acid dissolves in 256 parts water at 10° C. and in 18.6 parts boiling water, and on cooling crystallizes as rhombic prisms, and its 4 per cent solution acidified with HCl has the rotation $(\alpha)_D = +25.7°$; in alkaline solution the acid is levo-rotatory. It forms with copper oxide a crystalline compound which is soluble in boiling-hot water and nearly insoluble in cold water, and which may be used in the preparation of the pure acid from a mixture with other bodies.

The benzoyl-*l*-aspartic acid melts at 184-185°. For identification we make use of the analysis of the free acid and the copper salt, as well as of the specific rotation.

***d*-Glutamic acid** (α-aminoglutaric acid), $C_5H_9NO_4 = \begin{matrix}\dot{C}OOH,\\ \dot{C}H(NH_2)\\ \dot{C}H_2\\ \dot{C}H_2\\ \dot{C}OOH\end{matrix}$, is obtained from the protein substances under the same conditions as the other monamino-acids (see tables on pages 106, 107, 115 and 125) and from the peptones (SIEGFRIED). It is absent in the protamines and in the varieties of silk, it occurs only in small amounts with the exception of spider's web. HLASIWETZ and HABERMANN obtained 29 per cent from casein by cleavage with hydrochloric acid, while KUTSCHER could obtain only 1.8 per cent glutamic acid by cleavage with sulphuric acid. Other

[1] Ellinger, Ber. d. d. chem. Gesellsch., **37**; Neuberg and Silbermann, *ibid.*, **37**; Neuberg and Mayer, Biochem., Zeitschr **3**; Neuberg and Ascher, *ibid.*, **6**.

[2] Ber. d. d. chem. Gesellsch., **34**.

investigators such as ABDERHALDEN and FUNK and SKRAUP and TÜRK have shown that the same quantities of glutamic acid can be obtained by the use of the two mineral acids. SKRAUP and TÜRK obtained on the hydrolysis of casein 20.3–22.3 per cent glutamic acid hydrochloride corresponding to about 17 per cent glutamic acid. ABDERHALDEN and SASAKI [1] obtained 13.6 per cent glutamic acid from meat syntonin. It occurs most abundantly in the plant proteins where the quantity may be more than 40 per cent. LEVENE and MANDEL[2] have obtained a strikingly large quantity of glutamic acid, namely 25 per cent, from a nucleoprotein of the spleen.

On heating glutamic acid to 180–190° it is converted into pyrrolidon-carboxylic acid, which latter can be retransformed into glutamic acid by HCl gas; therefore, a formation of pyrrolidon-carboxylic acid at the same time, or in place of glutamic acid, in the hydrolyses, is not excluded.

On putrefaction glutamic acid gives γ-aminobutyric acid, *n*-butyric acid and succinic acid.

d-Glutamic acid crystallizes in rhombic tetrahedra or octahedra or in small leaves. It dissolves in 100 parts water at 16° C., and the solution has an acid taste with a peculiar after-taste. It is insoluble in alcohol and in ether.

In water it has a rotation of $(\alpha)_D = +12.04°$. Strong acids increase the rotation, and a 5 per cent solution of glutamic acid containing 9 per cent HCl has a rotation $(\alpha)_D = +31.7°$, while that obtained by heating with barium hydroxide is optically inactive. *d*-Glutamic acid forms a beautifully crystalline combination with hydrochloric acid, which is almost insoluble in concentrated hydrochloric acid. This compound is used in the isolation of glutamic acid. On boiling with cupric hydroxide a beautiful crystalline copper salt, which is soluble with difficulty, is obtained.[3] The benzoyl-*d*-glutamic acid melts at 130–132° C. The hydrochloride, the α-naphthylisocyanate of glutamic acid, which melts at 236–237° C., the analysis of the free acid, and the specific rotation are used in its detection.

As previously stated monamino-oxydicarboxylic acids have also been found among the cleavage products of the proteins. To these belong the following:

That **oxyaminosuccinic acid,** $C_{11}H_7NO_5$ occurs among the hydrolytic cleavage products of proteids has been shown to be probable by SKRAUP. This acid has

[1] Hlasiwetz and Habermann, Annal. d. chem. u. Pharm., **159**; Kutscher, Zeitschr., f. physiol. Chem., **28**; Abderhalden and Funk, *ibid.*, **58**; with Sasaki, *ibid.*, **51**; Skraup and Türk, Monatsch. f. Chem., **30**.

[2] Bioch. Zeitschr, **5**.

[3] Several salts of glutamic acid have been prepared and studied by Abderhalden and Kautzsch, Zeitschr. f. physiol. Chem., **64**, **68**, and **78**.

been prepared synthetically by NEUBERG and SILBERMANN from diaminosuccinic acid and barium nitrite in sulphuric acid solution. **Oxyaminosuberic acid**, $C_8H_{15}NO_5$, has been detected by WOHLGEMUTH [1] in the cleavage products of a liver nucleoprotein.

***l*-Cystine**, $C_6H_{12}N_2S_2O_4$ (α-diamino-β-dithiolactolic acid), the disulphide of **cysteine** (α-amino-β-thiolactic acid), $\begin{matrix}CH_2—S—S—CH_2\\ CH(NH_2) \quad CH(NH_2)\\ COOH \quad COOH\end{matrix}$, was first obtained, with certainty, as a cleavage product of protein substances by K. MÖRNER, and then also by EMBDEN. KÜLZ [2] obtained it once as a product of tryptic digestion of fibrin. The quantities found by MÖRNER and BUCHTALA in the various proteins are given in the tables on pages 106, 107, 115 and 125.

According to NEUBERG and MAYER[3] two kinds of cystine occur in nature, namely, *stone-cystine*, designated β-cystine, and *protein-cystine*, called α-cystine

Stone-cystine is the disulphide of β-amino-α-thiolactic acid, $\begin{matrix}CH_2NH_2 \quad CH_2NH_2\\ CH—S—S—CH\\ COOH \quad COOH\end{matrix}$

The protein-cystine has been chiefly obtained from the protein substance, but also from calculi, while the stone-cystine has been obtained from urinary calculi only.

Many objections have been raised from many sides as to the correctness of this assumption. ROTHERA could not find any difference between the stone-cystine and the cystine prepared from hair, and FISCHER and SUZUKI, and recently also ABDERHALDEN,[4] arrived at similar results, which seems to place the existence of stone-cystine in doubt. The occurrence of two structurally isomeric cystines is not improbable, from certain observations of MÖRNER, but FRIEDMANN and BAER[5] have shown that these observations do not lead to this assumption and at the present time we cannot admit of the occurrence of two different cystines.

Cystine probably occurs normally as traces in the urine. In rare cases, in cystinuria, it occurs in larger quantities in the urine, the sediment or in calculi. Traces have also been found in the ox-kidney, in the liver of the horse and dolphin, and in the liver of a drunkard. ABDERHALDEN [6] has found cystine in the urine and also abundantly in the organs (spleen) in a case of parental cystine diathesis.

The constitution of cystine has been explained by FRIEDMANN,[7] and

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[1] Skraup, Zeitschr. f. physiol. Chem., **42**; Neuberg and Silbermann, *ibid.*, **44**; Wohlgemuth, *ibid.*, **44**.

[2] K. Mörner, *ibid.*, **28**, **34**, and **42**; Embden, *ibid.*, **32**; Külz, Zeitschr. f. Biologie, **27**.

[3] Zeitschr. f. physiol. Chem., **44**.

[4] Rothera, Journ. of Physiol., **32**; Fischer and Suzuki, Zeitschr. f. physiol. Chem., **45**; Abderhalden, *ibid.*, **51**.

[5] Friedmann, Hofmeister's Beiträge, **3**. With Baer, *ibid.*, 8.

[6] Zeitschr. f. physiol. Chem., **38**.

[7] Hofmeister's Beiträge, **3**.

he has also established the relation between cystine and taurine. Cystine is the disulphide of cysteine, which is α-amino-β-thiolactic acid. From cysteine by oxidation FRIEDMANN obtained cysteinic acid, $C_3H_7NSO_5 = \begin{matrix}CH_2SO_2OH \\ CH(NH_2) \\ COOH\end{matrix}$, from which taurine $\begin{matrix}CH_2(SO_2OH) \\ CH_2(NH_2)\end{matrix}$ is produced by splitting off CO_2.

Cystine has also been prepared synthetically in several ways. For example, FISCHER and RASKE[1] have prepared cystine from α-amino-β-chlorpropionic acid (obtained from *l*-serine) by the action of barium hydrosulphide and a subsequent oxidation in the air.

l-Cystine crystallizes in thin, colorless, hexagonal plates. It is not soluble in water, alcohol, ether, or acetic acid, but dissolves in mineral acids and oxalic acid. It is also soluble in alkalies and ammonia, but not in ammonium carbonate. Cystine is optically active, being levorotatory. MÖRNER found it to be $(\alpha)_D = -224.3°$. On heating with hydrochloric acid it can, according to MÖRNER, be changed into a modification crystallizing in needles and with a weaker levorotatory power, or indeed dextrorotatory, composed of a mixture of the two optically active cystines. On heating with HCl to 165° for 12–15 hours NEUBERG and MAYER obtained inactive cystine. By fungus fermentation with Aspergillus niger they obtained dextrorotatory cystine. Cystine has no melting-point but slowly decomposes at 258–261°. On boiling cystine with caustic alkali it decomposes and yields alkali sulphide, which can be detected by lead acetate or sodium nitroprusside. According to MÖRNER[2] 75 per cent of the total sulphur is separated. Cystine treated with tin and hydrochloric acid develops only a little sulphureted hydrogen, and is converted into cysteine. Cystine yields sulphureted hydrogen and methyl mercaptan on putrefaction.

On heating upon platinum-foil cystine ignites and burns with a bluish-green flame, with the generation of a peculiar sharp odor. When warmed with nitric acid it dissolves with decomposition, and leaves on evaporation a reddish-brown residue, which does not give the murexid test.

Cystine is gradually precipitated from its sulphuric acid solution by phosphotungstic acid. Cystine forms crystalline salts with mineral acids and with bases. For isolating and separating cystine the precipitation with mercuric acetate is especially suited. The benzoyl cystine (BAUMANN and GOLDMANN[3]) melts at 180–181°; the phenylisocyanate compound at 160°. On boiling with 25 per cent hydrochloric acid this

[1] See Erlenmeyer and Stoop, Ber. d. d. chem. Gesellsch., **36**; Gabriel, *ibid.*, **38**; Fischer and Raske, *ibid.*, 41.

[2] Zeitschr. f. physiol. Chem., **34**.

[3] *Ibid.*, **12**.

compound passes to the anhydride, which is a hydantoin melting at 119° C. By the action of potassium cyanide MAUTHNER [1] obtained α-amino-β-suphocyanpropionic acid, $CH_2(SCN).CH(NH_2)COOH$.

Stone-cystine, according to NEUBERG and MAYER, differs in many respects from the ordinary cystine, among which the following may be mentioned: The optically active stone-cystine crystallizes in needles, the specific rotation is $(\alpha)_D = -206°$; it melts at 190–192° with marked swelling up. The benzoyl compound melts at 157–159°; the phenylcyanate compound melts at 170–172°, and it is not changed on boiling with hydrochloric acid,

In the detection and identification of cystine we make use of the crystalline form, the behavior on heating on platinum-foil, and the sulphur reaction after boiling with alkali. As to its preparation from protein substances see K. MÖRNER and FOLIN [2]. In regard to the detection of cystine in the urine see Chapter XIV.

Cysteine (α-amino-β-thiolactic acid), $C_3H_7NSO_2 = \begin{matrix}CH_2.SH\\ \dot{C}H(NH_2)\\ \dot{C}OOH\end{matrix}$, is formed from cystine by reduction with tin and hydrochloric acid. It is also produced in the cleavage of protein substances not as EMBDEN believes as a primary formation but according to MÖRNER and PATTEN [3] as a secondary formation. Cysteine can be easily converted into cystine by oxidation.

According to V. ARNOLD [4] cysteine occurs as a constituent of the press-juice or extracts of various animal organs. He has found it especially in the hair and he considers it as a primary cell constituent.

Toward alkalies and lead acetate it acts like cystine. With sodium nitroprusside and alkali it gives a deep purple-red coloration; with ferric chloride the solution gives an indigo-blue coloration which quickly disappears.

Thiolactic acid (α-thiolactic acid), $C_3H_6SO_2 = \begin{matrix}CH_3\\ \dot{C}H(SH)\\ \dot{C}OOH\end{matrix}$, has been found once as a cleavage product of ox-horn by BAUMANN and SUTER. MÖRNER, FRIEDMANN and BAER obtained it from cystine. It has been shown by FRIEDMANN that this acid is a regular cleavage product of keratin substances, and that it can also be obtained from the proteins. FRÄNKEL [5] obtained the acid from hæmoglobin. The pyroracemic acid obtained by MÖRNER as a decomposition product from several protein substances originates, according to MÖRNER, only in part from the cystine.

Taurine (aminoethylsulphonic acid), $C_2H_7NSO_3 = \begin{matrix}CH_2(NH_2)\\ \dot{C}H_2(SO_2.OH)\end{matrix}$, has not been obtained as a cleavage product of protein substances; still its

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[1] Zeitschr. f. physiol. Chem., **78**.

[2] Mörner, Zeitschr. f. physiol. Chem., **34**; Folin, Journ. of Biol. Chem., **8**.

[3] See foot-note 2, page 80.

[4] Zeitschr. f. physiol. Chem., **70**.

[5] Mörner, Zeitschr. f. physiol. Chem., **42**; Suter, Zeitschr. f. physiol. Chem., **20**; Friedmann, Hofmeister's Beiträge, **3**; with Baer, *ibid.*, 8; Fränkel, Sitzungsber. d. Wien. Akad., **112**, II, *b*, 1903.

origin from proteins has been shown by FRIEDMANN by the close relation that taurine bears to cysteine; and this is the reason why it is treated here in connection with the amino-acids.

Taurine is especially known as a cleavage product of taurocholic acid, and may occur to a slight extent in the intestinal contents. Taurine has also been found in the lungs and kidneys of oxen and in the blood and muscles of cold-blooded animals.

Taurine crystallizes in colorless, often in large, shining, 4- or 6-sided prisms. It dissolves in 15–16 parts of water at ordinary temperatures, but rather more easily in warm water. It is insoluble in absolute alcohol and ether; in cold alcohol it dissolves slightly, but better when warm. Taurine yields acetic and sulphurous acids, but no alkali sulphides, on boiling with strong caustic alkali. The content of sulphur can be determined as sulphuric acid after fusing with saltpeter and soda. Taurine combines with metallic oxides. The combination with mercuric oxide is white, insoluble, and is formed when a solution of taurine is boiled with freshly precipitated mercuric oxide (J. LANG [1]). This compound may be used in detecting the presence of taurine. Taurine is not precipitated by metallic salts.

The preparation of taurine from ox-bile is very simple. The bile is boiled a few hours with hydrochloric acid. The filtrate from the dyslysin and choloidic acid is concentrated well on the water-bath, and filtered hot so as to remove the common salt and other substances which have separated. The solution is evaporated to dryness and the residue dissolved in 5 per cent hydrochloric acid, and precipitated with 10 vols. 95 per cent alcohol. The crystals are readily purified by recrystallization from water.

The acid alcoholic solution can be used for the preparation of glycocoll. After the evaporation of the alcohol, the residue is dissolved in water, treated with a solution of lead hydroxide, filtered, the lead removed by H_2S, and the filtrate strongly concentrated. The crystals which separate are dissolved and decolorized by animal charcoal and the solution then evaporated to crystallization.

Though taurine shows no positive reactions, it is chiefly identified by its crystalline form, by its solubility in water and insolubility in alcohol, by its combination with mercuric oxide, by its non-precipitability by metallic salts, and above all by its sulphur content.

***l*-Phenylalanine** (phenyl-α-aminopropionic acid),

$$C_9H_{11}NO_2 = \begin{matrix} C_6H_5 \\ \dot{C}H_2. \\ \dot{C}H(NH_2), \\ \dot{C}OOH \end{matrix}$$

[1] See Maly's Jahresber, 6.

was first found by E. SCHULZE and BARBIERI [1] in etiolated lupin sprouts. It is produced in the acid cleavage of protein substances in quantities rarely above 5–6 per cent. It has been prepared synthetically in several ways by ERLENMEYER, JR., SÖRENSEN and E. FISCHER, WHEELER and HOFFMAN.[2]

The *l*-phenylalanine crystallizes in small, shining leaves or fine needles which are rather difficultly soluble in cold water but readily soluble in hot water. The solution has a faint bitter taste. A 5-per cent solution acidified with hydrochloric acid or sulphuric acid is precipitated by phosphotungstic acid, while a more dilute solution is not precipitated. On putrefaction, phenylalanine yields phenylacetic acid. On heating with potassium dichromate and sulphuric acid (25 per cent) an odor of phenylacetaldehyde is produced and benzoic acid is formed. In aqueous solution it has a rotation of $(\alpha)_D = -35.1°$. The phenylisocyanate-l-phenylalanine melts at about 182° C.

l-**Tyrosine** (*p*-oxyphenyl-α-aminopropionic acid),

$$C_9H_{11}NO_3 = \begin{matrix} C_6H_4(OH) \\ \dot{C}H_2 \\ \dot{C}H(NH_2) \\ \dot{C}OOH \end{matrix}'$$

is produced from most protein substances under the same conditions as leucine, which it habitually accompanies. The largest quantity of tyrosine obtained from animal proteins was about 10–13 per cent (see tables, pages 106, 107, 115 and 125). In gelatin and a few keratins tyrosine is absent. It is especially found with leucine, in large quantities, in old cheese (Τυρός), from which it derives its name. Tyrosine has not been found with certainty in perfectly fresh organs. It occurs in the intestine during the digestion of protein substances, and it has about the same physiological and pathological importance as leucine.

Tyrosine was prepared by ERLENMEYER and LIPP from *p*-aminophenylalanine by the action of nitrous acid, and according to another method by ERLENMEYER and HALSEY.[3] On fusing with caustic alkali it yields *p*-oxybenzoic acid, acetic acid, and ammonia. On putrefaction it may yield oxyphenylethylamine, oxyphenylpropionic acid, oxyphenylacetic acid, *p*-cresol and phenol.

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[1] Ber. d. d. chem. Gesellsch., **14**, and Zeitschr. f. physiol. Chem., **12**.

[2] Erlenmeyer, Annal. d. Chem. u. Pharm., **275**; Sörensen, Zeitschr. f. physiol. Chem., **44**; E. Fischer, Ber. d. d. chem. Gesellsch., **37**; Wheeler and Hoffman, Amer. Chem. Journ., **45**.

[3] Erlenmeyer and Lipp, Ber. d. d. chem. Gesellsch., **15**; Erlenmeyer and Halsey, *ibid.*, **30**.

Naturally occurring tyrosine and that obtained by the cleavage of protein substances by acids or enzymes, is generally *l*-tyrosine, while that obtained by decomposition with baryta-water or prepared synthetically is inactive. v. LIPPMANN [1] has obtained *d*-tyrosine from beet-sprouts. The statements as to specific rotation of tyrosine are somewhat variable. For tyrosine from proteins E. FISCHER has found a rotation of $(\alpha)_D = -12.56$ to 13.2° for the hydrochloric acid solution, while SCHULZE and WINTERSTEIN [2] obtained higher results using tyrosine from plants, namely, $(\alpha)_D = -16.2°$.

Tyrosine in a very impure state occurs in the form of balls similar to leucine. The purified tyrosine, on the contrary, appears as colorless, silky, fine needles which are often grouped into tufts or balls. It is difficultly soluble in water, being dissolved by 2454 parts of water at 20° C., and 154 parts boiling water, separating, however, as tufts of needles on cooling. It dissolves more easily in the presence of alkalies, ammonia, or a mineral acid. It is difficultly soluble in acetic acid. Crystals of tyrosine separate from an ammoniacal solution on the spontaneous evaporation of the ammonia. One hundred parts glacial acetic acid dissolve on boiling only 0.18 part tyrosine, and by this means, especially on adding an equal volume of alcohol before boiling, the leucine can be quantitatively separated from the tyrosine (HABERMANN and EHRENFELD [3]). The *l*-tyrosine-ethyl-ester crystallizes in colorless prisms which melt at 108–109° C. The naphthylisocyanate-*l*-tyrosine melts at 205–206°. Tyrosine can be oxidized with the formation of dark-colored products by various plant as well as animal oxidases, so-called tyrosinases (see Chapters XV and XVI). In alcoholic fermentation of sugar the tyrosine present at the same time is transformed according to F. EHRLICH [4] into *tyrosol* (*p*-oxyphenylethyl alcohol), $C_8H_{10}O_2$. Tyrosin is identified by its crystalline form and by the following reactions:

PIRIA'S *Test.* Tyrosine is dissolved in concentrated sulphuric acid by the aid of heat, by which tyrosine-sulphuric acid is formed; it is allowed to cool, diluted with water, neutralized by $BaCO_3$, and filtered. On the addition of a solution of ferric chloride the filtrate gives a beautiful violet color. This reaction is disturbed by the presence of free mineral acids and by the addition of too much ferric chloride.

HOFMANN'S *Test.* If some water is poured on a small quantity of tyrosine in a test-tube and a few drops of MILLON'S reagent added and

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[1] Ber. d. d. chem. Gesellsch., **17**.

[2] See Hoppe-Seyler-Thierfelder, Handb. d. physiol. u. pathol. chem. Analyse, 8. Aufl., 1909. Also E. Fischer, Ber. d. d. chem. Gesellsch., **32**; Schulze and Winterstein, Zeitschr. f. physiol. Chem., **45**.

[3] Zeitschr. f. physiol. Chem., **37**.

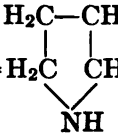
[4] Ber. d. d. chem. Gesellsch., **44**.

then the mixture boiled for some time, the liquid becomes a beautiful red and then yields a red precipitate.

DENIGÈS' *Test*, modified by C. MÖRNER, is performed as follows: To a few cubic centimeters of a solution consisting of 1 vol. formaline, 45 vols. water, and 55 vols. concentrated sulphuric acid add a little tyrosine in substance or in solution and heat to boiling. A beautiful permanent green coloration is obtained.

FOLIN and DENIS's *test*. The reagent consists of a solution containing 10 per cent sodium tungstate, 2 per cent phosphomolybdic acid and 10 per cent phosphoric acid. In performing the test mix 1–2 cc. of the reagent with an equal volume of the tyrosine solution and then add 3–10 cc. saturated sodium carbonate solution when a beautiful blue color results. Its delicacy is 1:1000000. The reagent can also be used for the colorimetric quantitative estimation of tyrosine in proteins. According to ABDERHALDEN and FUCHS and to ABDERHALDEN[1] the reagent suggested by FOLIN and DENIS for tyrosine also gives a blue coloration with tryptophane, oxytryptophane and *l*-oxyproline and the value of this reagent for quantitative tyrosine determinations requires further testing.

l-**Proline** (α-pyrolidine carboxylic acid), $C_5H_9NO_2 = \begin{array}{l} H_2C—CH_2 \\ \quad | \qquad\quad | \\ H_2C \quad CH.COOH, \\ \quad \diagdown \quad \diagup \\ \qquad NH \end{array}$



was first obtained by E. FISCHER and then by FISCHER and collaborators from several proteins as a primary cleavage product (ABDERHALDEN and KAUTZSCH[2]). The proline here obtained was generally the laevo-rotatory modification. The largest quantity of proline was secured from the vegetable proteins hordein and gliadin, namely, 13.7 per cent and 13.2 per cent, and also from gelatin, 7.7 per cent (see table pages 106, 107, 115 and 125). KOSSEL and DAKIN[3] obtained 11 per cent from salmine. Proline also occurs in scombrine and clupeine, but not in sturine, which, according to KOSSEL, seems to contradict the view as to the common origin of ornithine and proline.

SÖRENSEN,[4] by means of a general method of preparing α-amino-acids synthetically, has prepared α-amino-δ-oxyvaleric acid from phthalimidemalonic ester and has obtained proline from this by evaporating with

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[1] Denigès, Compt. rend., 130; C. Th. Mörner, Zeitschr. f. physiol. Chem., 37; Folin and Denis, Journ. of Biol. Chem., 12; Abderhalden and Fuchs, Zeitschr. f. physiol. Chem., 83 and Abderhalden, *ibid.*, 85.

[2] E. Fischer, Zeitschr. f. physiol. Chem., 33 and 35. See also footnote 2, p. 86, and Abderhalden and Kautzsch, Zeitschr. f. physiol. Chem., 78.

[3] Zeitschr. f. physiol. Chem., 41.

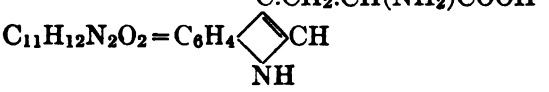
[4] Zeitschr. f. physiol. Chem., 44; with A. C. Anderson, *ibid.*, 56.

hydrochloric acid, at the same time splitting off water. Recently he has suggested another method which yields good results. Other syntheses of proline have also been performed by E. FISCHER and WILLSTÄTTER.[1] By the reduction of the ethyl ester of pyrrolidon carboxylic acid (see glutamic acid) E. FISCHER and BOEHNER[2] have obtained racemic α-proline. On putrefaction proline yields δ-amino-valeric acid and *n*-valeric acid (NEUBERG and ACKERMANN[3]).

l-Proline crystallizes in flat needles. It is readily soluble in water and alcohol. The solution has a sweet taste; the specific rotation at 20° C. is $(\alpha)_D = -77.40°$. The solution acidified with sulphuric acid is precipitated by phosphotungstic acid. In the detection of this acid we make use of the copper salt, the anhydride of the phenylisocyanate compound (melting-point 144°), and the picrate. The inactive acid and its compounds show somewhat different properties.

Oxyproline (oxy-α-pyrolidine carboxylic acid), $C_5H_9NO_3$. This acid, whose constitution is not understood was first obtained by E. FISCHER on the hydrolysis of casein and of gelatin. It dissolves readily in water; has a specific rotation of $(\alpha)_D = -81.04°$, and the solution has a sweet taste. Oxyproline crystallizes in beautiful colorless plates and gives a readily soluble copper salt. The constitution of natural oxyproline has recently been explained by LEUCHS and BREWSTER.[4] They find that the natural oxyproline is a γ-oxy-derivative of pyrrolidine-α-carboxylic acid. LEUCHS found the specific rotation of *l*-oxyproline to be $(\alpha)_D = -76°$ at 20° C.

***l*-Tryptophane** (indol-α-aminopropionic acid),



is one of the cleavage products of the proteins formed in tryptic digestion and other deep decompositions of the proteins, such as putrefaction, cleavage with baryta-water or sulphuric acid. It gives a reddish-violet product with chlorine or bromine which is called *proteinochrome*. NENCKI[5] considered tryptophane, which name is generally given to this acid, as the mother-substance of various animal pigments.

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[1] Ber. d. d. chem., Gesellsch., **33**.

[2] Ber. d. d. Chem., Gesellsch., **44**.

[3] Neuberg, Bioch. Zeitschr, **37**; Ackermann, Zeitschr. of Biol., **57**.

[4] Fischer, Ber. d. d. chem. Gesellsch., **35** and **36**; Leuchs and Brewster, Ber. d. d. Chem., Gesellsch., **46**.

[5] In regard to tryptophane, see Stadelmann, Zeitschr. f. Biologie, **26**; Neumeister, *ibid.*, **26**; Nencki, Ber. d. d. chem. Gesellsch., **28**; Beitler, *ibid.*, **31**; Kurajeff, Zeitschr. f. physiol. Chem., **26**; Klug, Pflüger's Arch., **86**.

Tryptophane was first prepared in a pure form by HOPKINS and COLE,[1] and they considered it as skatolaminoacetic acid. After ELLINGER showed that skatolcarbonic acid (SALKOWSKI) and skatolacetic acid (NENCKI) were indolacetic acid and indolpropionic acid respectively, and after the synthesis of *d-l*-tryptophane by ELLINGER and FLAMAND,[2] the nature of this substance as indolaminopropionic acid was established.

By condensation of β-indolaldehyde with hippuric acid ELLINGER and FLAMAND prepared the azlactone (lactimide):

$$C_8H_6NCHO+\overset{CH_2.NH.COC_6H_5}{\underset{COOH}{|}}=C_8H_6N.CH:\overset{N}{\underset{CO-O}{C\quad C}}.C_6H_5+2H_2O.$$

On boiling with dilute caustic soda, with the taking up of water, the sodium salt of indoxyl-α-benzoylaminoacrylic acid,

$$C_8H_6N.CH:\underset{COONa}{C.NH.COC_6H_5}$$

is obtained, from which by reduction and splitting off of the benzoyl group by the action of sodium alcoholate the tryptophane is obtained:

$$C_8H_6N.CH:\underset{COOH}{C.NH.COC_6H_5}+H_2+H_2O=C_8H_6N.CH_2.\underset{COOH}{CH.NH_2}+C_6H_5COOH.$$

The trytophane formed in digestion is *l*-tryptophane, which is lævorotatory in aqueous solution (HOPKINS and COLE). Racemic *d-l*-tryptophane has also been obtained by digestion in certain cases by ALLERS and NEUBERG, this is probably formed from the *l*-tryptophane (ABDERHALDEN and L. BAUMANN[3]), which very readily undergoes racemization.

Tryptophane crystallizes in silky rhombic or six-sided leaves. It does not have a sharp melting-point, and according to the rapidity of heating melts at 252°, 273° and 289°, according to various authorities. Tryptophane is readily soluble in hot water, difficultly soluble in cold water, and only slightly soluble in alcohol. The solution of *d-l*-tryptophane has a faintly sweetish taste, and *l*-tryptophane a faintly bitter taste. The statements as to the optical behavior of tryptophane differ somewhat, which, according to ABDERHALDEN, is probably due to the readiness with which it undergoes racemization. According to ABDERHALDEN and L. BAUMANN,[4] at 20° C. the aqueous solution has a rotation of

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[1] Journ. of Physiol., **27**.

[2] Ellinger, Ber. d. d. Chem. Gesellsch., **37** and **38**. With Flamand, *ibid.*, **40**, and Zeitschr. f. physiol. Chem., **55**.

[3] R. Allers, Biochem. Zeitschr., **6**; C. Neuberg, *ibid.*, **6**; Abderhalden and Baumann, Zeitschr. f. physiol. Chem., **55**. (Literature on the specific rotation.)

[4] See Abderhalden and Baumann, Zeitschr. f. physiol. Chem., **55** (literature).

$(\alpha)_D = -30.33°$. HOPKINS and COLE give $(\alpha)_D = -33°$ for the watery solution. It is dextrorotatory in $\frac{N}{1}$ or $\frac{N}{2}$ NaOH as well as in $\frac{N}{1}$ HCl.

Tryptophane yields indol and skatol when sufficiently heated. It gives the ADAMKIEWICZ-HOPKINS[1] reaction and a rose-red color on the addition of chlorine or bromine water (*tryptophane reaction*). The brom-tryptophane is readily soluble in amyl alcohol or acetic ether and on shaking with these solvents the reaction is more delicate.[2] If a pine stick previously moistened with hydrochloric acid and washed with water is introduced into a concentrated tryptophane solution, it becomes purple (pyrrole reaction) on drying. The melting-points of the benzoylsulphotryptophane, the β-naphthalenesulphotryptophane and the naphthylisocyanatetryptophane are according to ELLINGER and FLAMAND,[3] 185°, 180° and 158° C. respectively. Several compounds of tryptophane have been prepared by ABDERHALDEN and KEMPE.[4] Among these we will mention the tryptophane chloride hydrochloride, because it is used as the starting material for the synthesis of tryptophane polypeptides. In the alcoholic fermentation of sugar, as found by F. EHRLICH[5] the tryptophane present is transformed into *tryptophol* (β-indoxylethyl alcohol).

In regard to the rather complicated method for preparing tryptophane we must refer to the original work of HOPKINS and COLE, of NEUBERG, and of ABDERHALDEN and KEMPE. FASAL[6] has suggested a quantitative colorimetric method for estimating tryptophane based upon the ADAMKIEWICZ-HOPKINS reaction.

As shown by HOPKINS and COLE,[7] tryptophane on anaerobic putrefaction yields indolpropionic acid and indolacetic acid, and indol and skatol on aerobic putrefaction. Among these putrefactive products the indol and skatol will be specially discussed.

Indol, $C_8H_7N = C_6H_4$ CH, and **Skatol,** or β-METHYLINDOL,

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[1] In regard to this reaction see also Dakin, Journ. of Biol. Chem. 2, and O. Rosenheim, Biochem. Journ., 1.

[2] Neuberg, Bioch. Zeitschr., 24.

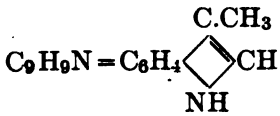
[3] l. c.

[4] Zeitschr. f. physiol. Chem., 52, and Ber. d. d. chem. Gesellsch., 40.

[5] Ber. d. d. chem., Gesellsch., 45.

[6] Hopkins and Cole, Journ. of Physiol., 27 and 29; Neuberg and Popowsky, Biochem. Zeitschr., 2; Abderhalden and Kempe, Zeitschr. f. physiol. Chem., 52; Fasal, Bioch. Zeitschr., 44.

[7] Journ. of Physiol., 29.



$C_9H_9N = C_6H_4$ $C.CH_3$ NH CH, are formed in variable quantities from protein compounds under different conditions. Hence they occur habitually in the human intestinal canal, and, after oxidation into indoxyl and skatoxyl respectively, pass, at least partly, into the urine as the corresponding ethereal sulphuric acids, and also as glucuronic acids.

Indol and skatol crystallize in shining leaves, and their melting-points are 52° and 95° C. respectively. Indol has a peculiar excrementitious odor, while skatol has an intense fetid odor. Both bodies are easily volatilized by steam, skatol more easily than indol. They may both be removed from the watery distillate by ether. Skatol is the more insoluble of the two in boiling water. Both are easily soluble in alcohol and give with picric acid a compound crystallizing in red needles. If a mixture of the two picrates be distilled with ammonia, they both pass over without decomposition; while if they are distilled with caustic soda, the indol but not the skatol is decomposed. The watery solution of indol gives with fuming nitric acid a red liquid and then a red precipitate of nitroso-indol nitrate (NENCKI [1]). It is better first to add two or three drops of nitric acid and then a 2-per cent solution of potassium nitrite, drop by drop (SALKOWSKI [2]). Skatol does not give this reaction. An alcoholic solution of indol treated with hydrochloric acid colors a pine chip cherry-red. Skatol does not give this reaction. Indol gives a deep reddish-violet color with sodium nitroprusside and alkali (LEGAL'S reaction). On acidifying with hydrochloric acid or acetic acid the color becomes pure blue. Skatol does not act the same. The alkaline solution is yellow and becomes violet on acidifying with acetic acid and boiling. With a few drops of a 4-per cent formaline solution and concentrated sulphuric acid indol gives a beautiful violet color while skatol gives a yellow or brown color (KONDO [3]). On warming skatol with sulphuric acid a beautiful purple-red coloration is obtained (CIAMICIAN and MAGNANINI [4]). According to SASAKI skatol, in methyl alcohol free from aldehyde, gives with concentrated sulphuric acid containing ferric salt a violet-red ring at the juncture of the two liquids. Indol and tryptophane do not give this reaction. DENIGÈS has carefully studied the behavior of these two bodies with EHRLICH'S reagent, dimethylaminobenzaldehyde, or with cinnamic aldehyde and vanillin. Comparative investigations on

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[1] Ber. d. d. deutsch. chem. Gesellsch., 8, 727, and *ibid.*, 722 and 1517.

[2] Zeitschr. f. physiol. Chem., 8, 447. In regard to newer reactions for indol and skatol, see Steensma, *ibid.*, 47, and Denigès, Compt. rend. soc. biol., 64.

[3] Zeitschr. f. physiol. Chem., 48.

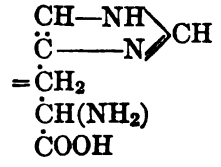
[4] Ber. d. d. chem. Gesellsch., 21, 1928.

the behavior of indol and skatol with the aromatic aldehydes have been carried out by BLUMENTHAL.[1]

For the detection of indol and skatol in, and their preparation from, fæces and putrefying mixtures, the main points of the usual method are as follows: The mixture is distilled after acidifying with acetic acid; the distillate is then treated with alkali (to combine with any phenols which may be present) and again distilled. From this second distillate the two bodies, after the addition of hydrochloric acid, are precipitated by picric acid. The precipitated picrate is then distilled with ammonia. The two bodies are obtained from the distillate by repeated shaking with ether and evaporation of the several ethereal extracts. The residue, containing indol and skatol, is dissolved in a very small quantity of absolute alcohol and treated with 8–10 vols. of water. Skatol is precipitated, but not the indol. The further treatment necessary for their separation and purification will be found in other works.[2]

Skatosine, $C_{10}H_{16}N_2O_3$, is a base first obtained by BAUM in the pancreas autodigestion and later studied by SWAIN. It develops an indol- or skatol-like odor on fusing with potassium hydroxide. LANGSTEIN [3] obtained a substance which is perhaps identical with skatosine, in the very lengthy peptic digestion of blood proteins.

***l*-Histidine,** $C_6H_9N_3O_2$, is β-imidazol-α-aminopropionic [4] acid,



Histidine was first discovered by KOSSEL in the cleavage products of sturine. It was found at the same time by HEDIN in the cleavage products of proteins by acid hydrolysis, and by KUTSCHER among the products of tryptic digestion, and finally also as a cleavage product of many different animal and plant protein substances. It does not occur in the protamines, with the exception of sturine. Of the protein bodies globin (from horse-hæmoglobin) seems to be richest in histidine, as

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[1] Sasaki, Bioch. Zeitschr. **23**, 29; Deniges, Compt. rend. soc. biol., **64**; Blumenthal, Bioch. Zeitschr., **19**.

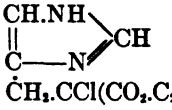
[2] For quantitative, colorimetric determinations of indol in feces see Einhorn and Hübner, Salkowski's Festschrift, Berlin, 1904; C. A. Herter and Foster, Journ. of biol. Chem., **2**.

[3] Baum, Hofmeister's Beiträge, **3**; Swain, *ibid.*; Langstein, see Hofmeister, Ueber Bau und Gruppierung der Eiweisskörper, in Ergebnisse der Physiologie, I, Abt. 1, 1902.

[4] See Pauly, Zeitschr. f. physiol. Chem., **42**; Knoop and Windaus, Hofmeister's Beiträge, **7** and **8**; Knoop, *ibid.*, **10**; Ackermann, Zeitschr. f. physiol. Chem., **65**.

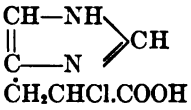
ABDERHALDEN found 10.96 per cent. It also occurs in germinating plants (E. SCHULZE [1]).

Histidine has been prepared synthetically by PYMAN.[2] 4 (5) chlormethyl glyoxalin yields with sodium chlormalonic ester the glyoxalinmethylchlormalonic ester.



, which on

hydrolysis gives *d-l-a*-chlor-*β*-glyoxalin-4 (5) propionic acid,



This latter treated with NH_3 yields *d-l*-histidine, which is changed into the active forms by means of tartaric acid.

In the anaerobic putrefaction of histidine, *β*-imidazolylethylamine and imidazolylpropionic acid are formed (ACKERMANN [3]).

Histidine crystallizes in colorless needles and plates and is readily soluble in water, but less soluble in alcohol, and has an alkaline reaction. It is precipitated by phosphotungstic acid, but this precipitate is soluble in an excess of the precipitant (FRÄNKEL). With silver nitrate alone the aqueous solution is not precipitated; on the careful addition of ammonia or baryta-water an amorphous precipitate, which is readily soluble in an excess of ammonia, is obtained. Histidine can be precipitated by mercuric chloride, or, still better, by the sulphate acidified with sulphuric acid, and can in this way be separated from the other diamino-acids (KOSSEL and PATTEN). The hydrochloride crystallizes in beautiful plates (BAUER), dissolves rather readily in water, but is insoluble in alcohol and ether. With hydrochloric acid and methyl alcohol it gives the dihydrochloride of histidine methyl ester, which melts at 196°. Histidine is lævorotatory, $(\alpha)_D = -39.74°$, while its solution in hydrochloric acid is dextrorotatory. On warming it gives the biuret test (HERZOG), and it also gives WEIDEL'S reaction if performed as suggested by FISCHER (see Xanthine, Chapter V) (FRÄNKEL [4]). On adding

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[1] Kossel, Zeitschr. f. physiol. Chem., **22**; Hedin, *ibid.*, Kutscher, *ibid.*, **25**; Wetzel, *ibid.*, **26**; Lawrow, *ibid.*, **28**, and Ber. d. d. chem. Gesellsch., **34**; Kossel and Kutscher, Zeitschr. f. physiol. Chem., **31**; Hart, *ibid.*, **33**; Abderhalden, *ibid.*, **37**; Schulze, *ibid.*, **24** and **28**.

[2] Cited from Chem. Centralbl., 1911, **2**, p. 760.

[3] Zeitschr. f. physiol. Chem., **65**.

[4] Kossel and Patten, Zeitschr. f. physiol. Chem., **38**; Bauer, *ibid.*, **22**; Herzog, *ibid.*, **37**; Fränkel, Sitz.-Ber. d. Wien. Akad., **112**, II. B., 1903, and Hofmeister's Beiträge, **8**.

sufficient bromine water and warming, a reddish coloration ensues which turns deep wine-red, later becoming cloudy, due to the formation of dark amorphous particles (F. KNOOP [1]). It gives a very beautiful diazo-reaction with diazobenzenesulphonic acid, in solutions made alkaline with sodium carbonate, which according to PAULY is deep cherry-red in dilutions of 1:20000 and still markedly red in 1:100000 (tyrosine gives a similar reaction).

Several salts of histidine are known; H. PAULY [2] has especially studied the iodized derivatives of histidine and imidazole.

On feeding *d-l*-histidine to rabbits ABDERHALDEN and WEIL [3] obtained from the urine *d*-histidine which was crystalline, was as sweet as sugar and showed a specific rotation $(\alpha)_D = +40.15°$ at 20° C.

Histidine is sometimes classified in a group, with the two diamino-acids, arginine and lysine which KOSSEL has called the *hexone bases*.

d-**Arginine** (δ-guanido-α-aminovaleric acid),

$$C_6H_{14}N_4O_2 = \begin{matrix} (HN)C{<}^{NH_2}_{NH.CH_2} \\ (CH_2)_2 \\ CH(NH_2) \\ COOH \end{matrix},$$

first discovered by SCHULZE and STEIGER in etiolated lupin- and pumpkin-sprouts, has later been found in other germinating plants, in tubers and roots. GULEWITSCH has found arginine in the ox-spleen, and TOTANI and KATSUYAMA have found it in ox-testicles. It was first found by HEDIN as a cleavage product of horn substance, gelatin, and several proteins, and then by KOSSEL and his pupils as a general cleavage product of protein substances as a class. The greatest quantity was obtained from the protamines; but the histones and certain plant proteins, edestin and the protein from pine seeds and especially excelsin (14.14 per cent), also yield abundant arginine. Arginine also occurs among the products of tryptic digestion (KOSSEL and KUTSCHER [4]).

On boiling with baryta-water, as well as by the action of an enzyme, *arginase*, discovered bv KOSSEL and DAKIN,[5] arginine yields urea and ornithine.

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[1] Hofmeister's Beiträge, 11.

[2] Ber. d. d. chem. Gesellsch., 42.

[3] Zeitschr. f. physiol. Chem., 77.

[4] Schulze and Steiger, Zeitschr. f. physiol. Chem., 11; Schulze and Castoro, *ibid.*, 41; Gulewitsch, *ibid.*, 30; Totani and Katsuyama, *ibid.*, 64; Hedin, *ibid.*, 20 and 21; Kossel and Kutscher, *ibid.*, 22, 25, 26.

[5] Zeitschr. f. physiol. Chem., 41, and Dakin, Journ. of biol. Chem., 8.

Arginine has been prepared synthetically from ornithine (α-δ-diaminovaleric acid) and cyanamide by SCHULZE and WINTERSTEIN. Recently SÖRENSEN and HÖYRUP[1] have prepared *d-l*-arginine from ornithuric acid. The α-monobenzoyl ornithine obtained by splitting ornithuric acid with $\frac{N}{5}$ barium hydrate yields α-benzoylamino-δ-guanido-valeric acid with cyanamide and this on boiling with hydrochloric acid gave δ-guanido-α-aminovaleric acid (*d-l*-arginine).

Arginine crystallizes in rosette-like tufts, plates, or thin prisms, is readily soluble in water with alkaline reaction and almost insoluble in alcohol. With several acids and metallic salts it forms crystalline salts and double salts respectively. Its acidified watery solution is precipitated by phosphotungstic acid. The most important salts are the copper-nitrate $(C_6H_{14}N_4O_2)_2.Cu(NO_3)_2+3H_2O$ and the silver salts

$$C_6H_{14}N_4O_2.HNO_3+AgNO_3$$

(the more readily soluble) and $C_6H_{14}N_4O_2.AgNO_3+\frac{1}{2}H_2O$ (the more difficultly soluble), and its compound with picrolonic acid (STEUDEL[2]).

Arginine is dextrorotatory. For arginine-chloride in watery solution with excess of hydrochloric acid, GULEWITSCH[3] found $(\alpha)_D=+21.25°$ at 20° C. The arginine obtained by KUTSCHER in the tryptic digestion of fibrin was racemic arginine. As found by KOSSEL and WEISS (see page 112) arginine or more properly the ornithine is very easily racemerized within the protein molecule by the action of alkali. The racemic arginine can, as RIESSER[4] has shown, during cleavage by means of arginase, yield *l*-arginine, which is an asymmetric change. In putrefaction arginine yields ornithine, guanidine, putrescine and δ-aminovaleric acid.

Agmatine (guanidobutylamine), $C_5H_{14}N_4=HN.C\begin{cases}NH_2\\NH.CH_2(CH_2)_2.CH_2NH_2\end{cases}$, is a base obtained by KOSSEL in the hydrolysis of herring sperm, and later by KUTSCHER and ENGELAND[5] from ergot. KOSSEL has also obtained it synthetically from cyanamide and tetramethylendiamine, and in this manner proved its constitution. It is produced from arginine by splitting off CO_2 and bears the same relation to arginine that putrescine does to ornithine and cadeverine does to lysine (see below). Agmatine gives several crystalline salts as described by KOSSEL. It is precipitated by phosphotungstic acid.

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[1] Schulze and Winterstein, Ber. d. d. chem. Gesellsch., **32** and Zeitschr. f. physiol. Chem., **34**; Sörensen and Höyrup, Ber. d. d. chem. Gesellsch, **43** and Zeitschr. f. physiol. Chem., **76**.

[2] Zeitschr. f. physiol. Chem., **37** and **44**.

[3] *Ibid.*, **27**.

[4] Kutscher, Zeitschr. f. physiol. Chem., **28** and **32**; Riesser, *ibid.*, **49**.

[5] Kossel, *ibid.*, **66** and **68**; Engeland and Kutscher, Centralbl. f. Physiol., **24**, **479**.

d-**Ornithine** (α-δ-diaminovaleric acid), $C_5H_{12}N_2O_2 = \begin{matrix}CH_2.(NH_2)\\(CH_2)_2\\CH(NH_2)\\COOH\end{matrix}$, is not a primary cleavage product of proteins, but is formed from arginine on boiling with baryta-water. JAFFE,[1] who first discovered this body, obtained it as a cleavage product from ornithuric acid, which is found in the urine of hens fed with benzoic acid. The ornithine which E. FISCHER and later SÖRENSEN,[2] have prepared synthetically yields, as shown by ELLINGER, putrescine (tetramethylenediamine), $C_4H_8(NH_2)_2$, on putrefaction. A. LOEWY and NEUBERG[3] have shown that ornithine is split into putrescine and CO_2 in the organism of cystinuria patients.

Ornithine is a non-crystalline substance which dissolves in water, giving an alkaline reaction, and yields several crystalline salts. It is precipitated by phosphotungstic acid and several metallic salts, but not by silver nitrate and baryta-water (differing from arginine). Ornithine hydrochloride is dextrorotatory; the synthetically prepared one is inactive. On shaking ornithine with benzoyl chloride and caustic soda it is converted into dibenzoylornithine (ornithuric acid). On splitting artificially prepared racemic ornithuric acid SÖRENSEN has shown that the naturally occurring ornithuric acid is identical with the dextrorotatory α-δ-dibenzoyldiaminovaleric acid. Salts and derivatives of ornithine have been described by KOSSEL and his collaborators[4] and they have given a method for its isolation from mixtures.

Diaminoacetic acid, $C_2H_6N_2O_2 = CH(NH_2)_2COOH$ was obtained by DRECHSEL[5] as a cleavage product of casein by boiling with tin and hydrochloric acid. It crystallizes in prisms and gives a monobenzoyl compound which is not very soluble in cold water and is almost insoluble in alcohol, and can be used in the isolation of the acid.

d-**Lysine** (α-ϵ-diaminocaproic acid), $C_6H_{14}N_2O_2 = \begin{matrix}CH_2(NH_2)\\(CH_2)_3\\CH(NH_2)\\COOH\end{matrix}$, was first obtained by DRECHSEL as a cleavage product of casein. Later he and his pupils, as well as KOSSEL and others, found it among the cleavage products of various proteins. It has not been detected in some vegetable proteins such as the prolamines (page 106). E. SCHULZE found lysine in germinating plants of the Lupinus luteus, and WINTERSTEIN found it in ripe cheese. It has been obtained in largest amounts (28.8 per cent) by KOSSEL and DAKIN from the protamine α-cyprinine. From a gliadin which was not contaminated and which they considered as a unit substance although obtained from different fractions having different solubilities in alcohol, OSBORNE and LEAVENWORTH[6] found a small amount of lysine

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[1] Ber. d. d. chem. Gesellsch., **10** and **11**.

[2] Fischer, Ber. d. d. chem. Gesellsch, **34**; Sörensen, Zeitschr. f. physiol. Chem., **44**.

[3] Ellinger, Zeitschr. f. physiol. Chem., **29**; Loewy and Neuberg, *ibid.*, **43**.

[4] Kossel and Weiss, Zeitschr. f. physiol. Chem., **68**.

[5] Ber. d. k. sächs. Gesellsch. d. Wiss., **44**

[6] Drechsel, Arch. f. (Anat. u.) Physiol., 1891, and Ber. d. d. chem. Gesellsch., **25**; Siegfried, Arch. f. (Anat. u.) Physiol., 1891, and Ber. d. d. chem. Gesellsch., **24**; Hedin, Zeitschr. f. physiol. Chem., **21**; Kossel, *ibid.*, **25**; Kossel and Mathews, *ibid.*, **25**; Kossel

(0.07 and 0.15 per cent in two different fractions). The generally accepted view that lysine is completely absent in gliadin is still doubtful. They could not detect lysine in zein by the same method.

Lysine has been synthetically prepared by E. FISCHER and WEIGERT.[1] This lysine was racemic, while that prepared from protein is always optically active and dextrorotatory. The rotation depends upon the concentration and degree of acidity; for the hydrochloride a rotation of $(\alpha)_D = +14°$ to 17.25° has been found. On heating with barium hydroxide it is converted into the racemic modification. According to ELLINGER lysine yields cadaverine (pentamethylenediamine), $C_5H_{10}(NH_2)_2$, on putrefaction, and this base is formed from the lysine in the organism of those with cystinuria and at the same time CO_2 is split off (A. LOEWY and NEUBERG).[2]

Lysine is readily soluble in water but is not crystallizable. The aqueous solution is precipitated by phosphotungstic acid, but not by silver nitrate and baryta-water (differing from arginine and histidine). It gives two hydrochlorides with hydrochloric acid, and with platinum chloride a chloroplatinate which is precipitable by alcohol and has the composition $C_6H_{14}N_2O_2.H_2PtCl_6+C_2H_5OH$. It gives two silver salts with $AgNO_3$; one has the formula $AgNO_3+C_6H_{14}N_2O_2$ and the other $AgNO_3+C_6H_{14}N_2O_2.HNO_3$. With benzoyl chloride and alkali, lysine forms an acid, *lysuric acid*, $C_6H_{12}(C_7H_5O)_2N_2O_2$ (DRECHSEL), which is homologous with ornithuric acid, and whose difficultly soluble acid barium salt may be used in the separation of lysine.[3] The rather insoluble picrate, which is precipitated from a not too dilute solution of the hydrochloride by sodium picrate, may also be used in the detection of lysine.

KUTSCHER and LOHMANN [4] have found a lysine having somewhat different properties in the final products of pancreas autolysis.

In the preparation of the so-called hexone bases we can first precipitate all the bases by phosphotungstic acid, when the monamino-acids remain in solution. The precipitate is then decomposed in boiling water by barium hydroxide and the bases obtained as silver compounds from this filtrate. In regard to further details and the methods of separating the various

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and Kutscher, *ibid.*, **31**; Kutscher, *ibid.*, **29**; Schulze, *ibid.*, **28**; Winterstein, cited in Schulze and Winterstein, Ergebnisse der Physiologie, I, Abt. 1, 1902; Kossel and Dakin, Zeitschr. f. physiol. Chem., **40**; Osborne and Leavenworth, Journ. of biol. Chem., **14**

[1] Ber. d. d. chem Gesellsch., **35**.

[2] See footnote 3, p. 163.

[3] Drechsel, Ber. d. d. chem. Gesellsch., **28**; see also C. Willdenow, Zeitschr. f. physiol. Chem., **25**.

[4] Zeitschr. f. physiol. Chem., **41**.

bases we will refer to STEUDEL in ABDERHALDEN's Handbuch der biochemischen Arbeitsmethoden, Bd. 2, II, s. 498.

We give below a tabulation of the amounts of the three hexone bases found in certain protein substances (in weight per cent):

	Arginine.	Lysine.	Histidine.
Sturine [1]	58.2	12.0	12.9
Cyprinine (α) [5]	4.9	28.8	0.0
Other protamines [1]	62.5 —87.4	0.0	0.0
Histones [1]	14.36—15.52	7.7 —8.3	1.21—2.34
Casein [2]	4.70— 4.84	1.92—5.80	2.53—2.59
Syntonin (from meat) [2]	5.06	3.26	2.66
Heterosyntonose [2]	8.53	3.08—7.03	0.37—1.12
Protosyntonose [2]	4.55	3.08	3.35
Edestin [3]	11.0—14.07	1.3	1.17
Proteid from coniferæ seeds [3]	10.9—11.3	0.25—0.79	0.62—0.78
Gluten casein [1]	4.4	2.15	1.16
Gluten proteins [1]	2.75— 3.13	0.0	0.43—1.53
Gelatin [1] and [2]	7.62— 9.3	2.49—6.0	0.40
Elastin [4]	0.3	+	0.027

Of the oxydiamino-acids found on the hydrolysis of proteins we will mention the following:

Oxydiaminosebacic acid, (?) $C_{10}H_{20}N_2O_5$, has been isolated by WOHLGEMUTH [6] from a nucleoprotein of the liver. The free acid was obtained as small white plates. It is soluble with difficulty in hot water, insoluble in cold water and in alcohol. It was optically inactive in hydrochloric acid. The beautifully crystalline phenylcyanate compound had a melting-point of 206°.

Dioxydiaminosuberic acid, $C_8H_{16}N_2O_6$, has been obtained by SKRAUP [7] on the hydrolysis of casein with hydrochloric acid. The copper salt crystallizes in beautiful deep bluish-violet rosettes which are composed of long, irregular, right-angled plates. It is quite soluble in cold water. The free acid crystallizes in fern-like formations. Besides this acid SKRAUP obtained two other acids which he calls *caseanic acid*, $C_8H_{16}N_2O_7$, and *caseinic acid*, $C_{12}H_{24}N_2O_6$. The caseanic acid crystallizes, melts at 190–191°, is tribasic, and is probably an oxydiamino-acid. The caseinic acid is dibasic and occurs in two modifications. The one which melts at 228° is faintly dextrorotatory; the other modification melts at 245° and is optically inactive. Both crystallize, but the inactive form does not yield well-defined crystals. Caseinic acid seems also to be an oxydiamino-acid.

Diaminotrioxydodecanoic acid, $C_{12}H_{26}N_2O_5$, is an acid obtained by FISCHER and ABDERHALDEN [8] on the hydrolysis of casein and seems to stand close to SKRAUP's caseinic acid, but differs from it in its optical properties. This acid is faintly levorotatory: $(\alpha)_D$ = about −9°. It crystallizes in plates, which grow into rosettes

[1] Kossel and Kutscher, Zeitschr. f. physiol. Chem., **31**.

[2] Hart, *ibid.*, **33**.

[3] Schulze and Winterstein, *ibid.*, **32**; see also Kossel, Ber. d. d. chem. Gesellsch., **34**, 3236.

[4] Kossel and Kutscher, Zeitschr. f. physiol. Chem., **25**, and Richards and Gies, Amer. Journ. of Physiol., **7**.

[5] Kossel and Dakin, Zeitschr. f. physiol. Chem., **40**.

[6] Ber. d. d. chem. Gesellsch., **37**, and Zeitschr. f. physiol. Chem., **44**.

[7] Zeitschr. f. physiol. Chem., **42**.

[8] Zeitschr. f. physiol. Chem., **42**.

or spherical aggregations. It has a faint bitter taste, gives a crystalline hydrochloride which is slightly soluble in strong hydrochloric acid, and gives a crystalline copper salt.

After describing the different amino-acids it remains for us to call attention to certain general reactions of the amino-acids.

By the action of formaldehyde the amino groups are changed into methylene groups according to the scheme:

$$\begin{matrix} R.CH.NH_2 \\ | \\ COOH \end{matrix} + HCOH = \begin{matrix} R.CH.N : CH_2 \\ | \\ COOH \end{matrix} + H_2O.$$

The amino-acids behave like neutral bodies while the methylene combinations are acids and on this behavior is based SÖRENSEN'S [1] formoltitration which serves for the estimation of amino-acids in the urine (Chapter XIV) as well as to follow the progress of proteolysis. As the proteolysis progresses and imide bindings are loosened a large number of atomic complexes with free NH_2 and COOH groups are set free. If now the NH_2 groups are fixed as methylene groups by the addition of formol, the complex behaves like acids and the number of their COOH groups can be determined by titration with $\frac{N}{5}$ barium or sodium hydroxide solution, using phenolphthalein or thymolphthalein as indicator. With the presumption that for every COOH group set free there existed a free NH_2 group the extent of the proteolysis can also be expressed in milligrams N by multiplying the number of cubic centimeters $\frac{N}{5}$ alkali used by 2.8.

SIEGFRIED has found that amino-acids in the presence of alkali or alkaline earths de-ionize carbon dioxide and form salts of the type of the carbamino salts, SIEGFRIED'S "carbamino-reaction." For example glycocoll in the presence of lime yields with carbon dioxide, calcium carbamino-acetic acid, $\begin{matrix} CH_2.NH.COO \\ | \quad\quad | \\ COO\text{———}Ca \end{matrix}$.

If the nitrogen is determined and at the same time the combined carbon dioxide estimated by means of the calcium carbonate split off on boiling the filtered solution, then the quotient $\frac{CO_2}{N}$ gives the number of N atoms for every molecule CO_2 taken up. This quotient is equal to 1 for glycocoll and the aliphatic amino-acids because these go over quan-

[1] Sörensen, Bioch. Zeitschr., 7; with Jessen Hansen, *ibid.*, 7; with V. Henriques, Zeitschr. f. physiol. Chem., 63 and 64; Henriques and Gjaldbäk, *Ibid.*, 67 and 75.

titatively into carbamino-acids. With the diamino-acid arginine, which contains 4 nitrogen atoms, it is on the contrary only one-fourth because this acid reacts with only one amino group, that of the α-amino valeric acid chain.

The reaction which has been developed and extensively used by SIEGFRIED [1] and his pupils is of great value in the characterization of peptones, kyrines, and proteoses, for the separation and fractional precipitation and for the determination of their constitution. The binding of the carbon dioxide as carbamino-salts seems also in many ways to be of physiological importance, as for example, the solubility of calcium carbonate in alkaline fluids and for the carbon dioxide binding in blood, etc.

The amino-acids can by methylation form betaines, for example, trimethyl glycocoll or betaine $\begin{matrix} CH_2—N(CH_3)_3 \\ | \quad | \\ CO—O \end{matrix}$. Betaine occurs abundantly in the plant kingdom. In the animal kingdom such bodies have been found under physiological conditions in cold blooded animals and they belong to those groups of bodies which have been called "aporrhegmas" by ACKERMANN and KUTSCHER.[2] As "aporrhegmas" they designate all those fractions of amino-acids from the protein, which can be produced from the proteins in a physiological manner and indeed in the life of animals as well as the plants. These bodies are essentially the same as have been observed in the putrefaction of the amino-acids and which have been specially mentioned with every amino-acid described.

The behavior of the amino-acids in yeast fermentation will be discussed in Chapter III.

In regard to the methods for separating and preparing, in a pure form, the various amino-acids and other products of protein hydrolysis which have not been given in the preceding pages, we must refer to ABDERHALDEN'S Handbuch der biochemischen Arbeitsmethoden, 1909–1910 Bd. 2.

II. Compound Proteins.[3]

We designate as compound proteins those bodies which yield, on cleavage, proteins (with their decomposition products) and other bodies such as carbohydrates, nucleic acids, or pigments.

The compound proteins known at present can be divided into three groups: *glycoproteins*, *nucleoproteins* and *chromoproteins*. Of these the

[1] In regard to the literature see Siegfried in Ergebnisse d. Physiol. Bd. 9.

[2] Zeitschr. f. physiol. Chem., **69**. See also Engeland, *ibid.*, **69**.

[3] Hoppe-Seyler has given the name *proteide* to these compound proteids, but as this term is misleading in English we do not use it in English classifications in this sense.

last-mentioned group (hæmoglobin and hæmocyanine) will be discussed in a subsequent chapter (Chapter V on the blood).

A. Glycoproteins (glucoproteins).

Glycoproteins[1] are those compound proteins which on decomposition yield a protein on the one side, and a carbohydrate or derivatives of this on the other, but no purine bodies. Some glycoproteids are free from phosphorus (mucin substances, chondroproteins, and hyalogens), and some contain phosphorus (phosphoglycoproteins).

The glycoproteins free from phosphorus may, as regards the nature of the carbohydrate groups split off, be divided into two chief groups, the *mucin substances* and the *chondroproteins*. The first yield on hydrolytic cleavage an amino-sugar, which has been shown to be glucosamine in all but a few exceptions.[2] In the chondroproteins, on the contrary, the protein is united to chondroitin-sulphuric acid.

1. Mucin Substances.

Compared with the simple proteins the mucin substances are poorer in nitrogen and as a rule also have considerably less carbon. The carbohydrate complex, whose nature has been shown by the investigations of Fr. Müller[3] and his pupils, occurs, so it seems, in the mucin substances as a polysaccharide related to chitosan, which on hydrolytic cleavage yields glucosamine (chitosamine), and, at least in most cases, acetic acid also. The mucin substances differ very markedly among themselves, hence we divide them into two groups, the mucins and the mucoids.

The *true mucins* are characterized by the fact that their natural

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[1] Abderhalden (Lehrb d. physiol. Chem., 1909, p. 191) has proposed dropping the name glycoproteids entirely and to consider these bodies as simple proteins, because it has not been shown that the carbohydrate groups occupy the same relationship to the protein component that the hæmin or the nucleic acid bears to the hæmoglobin or the nucleoprotein molecule. It is possible that this proposition, which is not applicable to the entire group (including the proteins containing chondroitin-sulphuric acid) but applies only to the mucin group, will be found in the future to be correct. It is the opinion of Hammarsten that it is better to wait for further research in this direction before we drop the generally accepted nomenclature and the usual subdivisions of the proteins.

[2] See Schulz and Ditthorn, Zeitschr. f. physiol. Chem., 29; A. v. Ekenstein and Blanksma, Chem. Centralbl., 1907, 2. When both carbohydrate groups are simultaneously combined in one body, then probably we are not dealing with a chemical individual, but rather with a mixture.

[3] See Fr. Müller, Zeitschr. f. Biologie, 42, which contains all the pertinent literature, and also L. Langstein, Die Bildung von Kohlenhydraten aus Eiweiss, Ergebnisse der Physiologie, Jahr. I, Abt. 1.

solutions, or solutions prepared by the aid of a trace of alkali, are mucilaginous, ropy, and give a precipitate with acetic acid which is insoluble in excess of acid or soluble only with great difficulty. The *mucoids* do not show these physical properties, and have other solubilities and precipitation properties. As we have intermediate steps between different protein bodies, so also we have such between true mucins and mucoids, and a sharp line cannot be drawn between these two groups.

It is just as difficult at present to draw a sharp line between the proteins and the mucins or mucoids, since we have been able to split off carbohydrate complexes from several proteins, and as proteins have been isolated from white of egg which yield more or less glucosamine. The very variable amounts of glucosamine obtained under various conditions from the crystalline ovalbumin seem to indicate that we are dealing with a contamination with a glycoprotein.

True mucins are secreted by the larger mucous glands, by certain mucous membranes, and by the skin of snails and other animals. True mucin also occurs in the navel-cord. Sometimes, as in snails and in the membrane of the frog-egg (GIACOSA) and perch-eggs (HAMMARSTEN [1]), a mother-substance of mucin, a mucinogen, has been found which may be converted into mucin by alkalies. Mucoid substances are found in certain cysts, in the cornea, the crystalline lens, white of egg, and in certain ascitic fluids. The so-called tendon-mucin, which, according to the investigations of LEVENE and of CUTTER, and GIES,[2] contains chondroitin-sulphuric acid or a related substance, cannot be classified as a mucin, but must, like the chondromucoid and the osseomucoid, be classified as chondroprotein. As the mucin question has not been sufficiently studied, it is at the present time impossible to give any positive statements in regard to the occurrence of mucins and mucoids, especially as without doubt in many cases non-mucinous substances have been described as mucins.

True Mucins. Thus far we have been able to obtain only a few mucins in a pure and unchanged condition, because of the reagents used. The elementary analyses of these mucins have given the following results:

	C	H	N	S	
Mucin from mucous membrane (air-passages)	48.26	6.91	10.70	1.40	(FR. MULLER [3])
Mucin from submaxillary	48.84	6.80	12.32	0.84	(HAMMARSTEN [3])
Mucin from snail	50.32	6.84	13.65	1.75	(HAMMARSTEN [3])
Synovial mucin	51.05	6.53	13.01	1.34	(v. HOLST [4])

[1] Giacosa, Zeitschr. f. physiol. Chem., 7; Hammarsten, Pflüger's Archiv., 36, and Skand, Arch. f. Physiol., 17.

[2] Levene, Zeitschr. f. physiol. Chem., 31; Cutter and Gies, Amer. Journ. of Physiol., 6.

[3] Fr. Müller, Zeitschr. f. Biologie, 42; Hammarsten, Zeitschr. f. physiol. Chem.. 12, and Pflüger's Arch., 36.

[4] Zeitschr. f. physiol. Chem., 42.

MÜLLER obtained 35 per cent glucosamine from mucous-membrane mucin and 23.5 per cent from the submaxillary mucin.

On boiling mucin with dilute mineral acids, acid albuminate and bodies similar to proteoses are obtained, besides a reducing substance which is not free glucosamine (STEUDEL [1]). By the action of strong acids upon mucins or mucoids OTORI [2] obtained several of the cleavage products of the proteins, such as leucine, tyrosine, glycocoll, glutamic acid, oxalic acid, guanidine, arginine, lysine, and humus substances, and also carbohydrate cleavage products, such as levulinic acid. Certain mucins, as the submaxillary mucin, are easily changed by very dilute alkalies, as lime-water, while others, such as tendon-mucin, are not affected. If a strong caustic-alkali solution, such as a 5-per cent KOH solution, is allowed to act on submaxillary mucin, we obtain alkali albuminate, bodies similar to proteoses and peptones and one or more substances of an acid reaction which have strong reducing powers.

On peptic digestion proteoses and peptone-like bodies, still containing the carbohydrate group, are produced. On tryptic digestion still simpler cleavage products are formed, namely, leucine, tyrosine, and tryptophane (POSNER and GIES [3]). The glucosamine, so far as we know, is not split off by proteolytic enzymes, but only after strong hydrolysis with acids.

In one or another respect the various mucins act somewhat dissimilarly. For example, the snail and sputum mucins are insoluble in dilute hydrochloric acid of 1–2 p. m., while the mucin of the submaxillary gland and the navel-cord is soluble. The former become flaky with acetic acid, while the submaxillary mucin is precipitated in more or less fibrous, tough masses. Still all the mucins have certain reactions in common.

In the dry state mucin forms a white or yellowish-gray powder. When moist it forms, on the contrary, flakes or yellowish-white tough lumps or masses. The mucins are acid in reaction. They give the color reactions of the proteins. They are not soluble in water, but may give a neutral solution with water with the aid of small amounts of alkali. Such a solution does not coagulate on boiling, but acetic acid gives at the normal temperature a precipitate which is nearly insoluble in an excess of the precipitant. If 5–10 per cent NaCl be added to a mucin solution, it can be carefully acidified with acetic acid without giving a precipitate. Such acidified solutions are copiously precipitated by tannic acid; with potassium ferrocyanide they give no precipitate, but on sufficient concentration they become thick or viscous. A neutral solution of alkali mucin is precipitated by alcohol in the presence of neutral

[1] Zeitschr. f. physiol. Chem., **34**.
[2] *Ibid.*, **42** and **43**.
[3] Amer. Journ. of Physiol., **11**.

salts; it is also precipitated by several metallic salts. If mucin is heated on the water-bath with dilute hydrochloric acid of about 2 per cent, the liquid gradually becomes a yellowish or dark brown, and reduces copper salts in alkaline solutions.

The mucin most readily obtained in large quantities is the submaxillary mucin, which may be prepared in the following way: The filtered watery extract of the gland, free from form-elements and as colorless as possible, is treated with 25 per cent hydrochloric acid, so that the liquid contains 1.5 p. m. HCl. On the addition of the acid the mucin is immediately precipitated, but dissolves on stirring. If this acid liquid is immediately diluted with 2–3 vols. of water, the mucin separates and may be purified by redissolving in 1–5 p. m. acid, and diluting with water and washing therewith. The mucin of the navel-cord may be prepared in the same way. As a rule the mucins can be prepared by precipitation with acetic acid and repeated solution in dilute lime-water or alkali, and reprecipitation with acetic acid. Finally they are treated with alcohol and ether. In the preparation of sputum mucin the method is very complicated (FR. MÜLLER).

Mucoids or **Mucinoids.** In this group we must include those non-phosphorized glycoproteins which are neither true mucins nor chondroproteids, although they show among themselves such differences in behavior that they can be divided into several subgroups of mucoids. To the mucoids belong *pseudomucin*, the probably related body *colloid*, *ovomucoid*, and other bodies, which on account of their differences will be best treated individually in their respective chapters.

Hyalogens. Under this name KRUKENBERG [1] has designated a number of different bodies, which are characterized by the following: By the action of alkalies they change, with the splitting off of sulphur and some nitrogen, into soluble nitrogenized products called by him *hyalines*, and which yield a pure carbohydrate by further decomposition. We find that very heterogeneous substances are included in this group. Certain of these hyalogens seem undoubtedly to be glycoproteins. *Neossin* [2] of the Chinese edible swallow's-nest, *membranin* [3] of DESCEMET'S membrane and of the capsule of the crystalline lens, and *spirographin* [4] of the skeletal tissue of the worm Spirographis, seem to act as such. Others, on the contrary, such as *hyalin* [5] of the walls of hydatid cysts, and *onuphin*,[6] from the tubes of Onuphis tubicola, do not seem to be compound proteins. The so-called *mucin of the holothuria* [7] and *chondrosin* [8] of the sponge, Chondrosia

[1] Verh. d. physik.-med. Gesellsch. zu Würzburg, 1883; also Zeitschr. f. Biologie, **22**.

[2] Krukenberg, Zeitschr. f. Biologie, **22**.

[3] C. Th. Mörner, Zeitschr. f. physiol. Chem., **18**.

[4] Krukenberg, Würzburg, Verhandl., 1883; also Zeitschr. f. Biologie, **22**.

[5] A. Lücke, Virchow's Arch., **19**; also Krukenberg, Vergleichende physiol. Stud., Series 1 and 2, 1881.

[6] Schmiedeberg, Mitth. aus d. zool. Stat. zu Neapel, **3**, 1882.

[7] Hilger, Pflüger's Archiv, **3**.

[8] Krukenberg, Zeitschr. f. Biologie, **22**.

reniformis, and others may also be classed with the hyalogens. As the various bodies designated by KRUNKENBERG as hyalogens are very dissimilar, it is not of much advantage to arrange these in special groups.

2. Chondroproteins.

Chondroproteins are those glycoproteins which as primary cleavage products yield protein and an ethereal sulphuric acid, the *chondroitin-sulphuric acid. Chondromucoid*, occurring in cartilage, is the best example of this group. *Amyloid* occurring under pathological conditions also belongs to this group. On account of the property of chondroitin-sulphuric acid of precipitating protein, it is also possible that under certain circumstances combinations of this acid with protein may be precipitated from the urine and be considered as chondroproteins.

The chondromucoid, the so-called tendon-mucin, and the osseomucoid have greatest interest as constituents of cartilage, of the connective tissues, and the bones, and on this account these bodies and their cleavage product, chondroitin-sulphuric acid, will be treated in a following chapter (IX). On the contrary, amyloid, which has always been considered in connection with the protein substances, will be described here.

Amyloid, so called by VIRCHOW, is a protein substance appearing under pathological conditions in the internal organs, such as the spleen, liver and kidneys, as infiltrations; and in serous membranes as granules with concentric layers. It probably also occurs as a constituent of certain prostate calculi. The chondroprotein occurring under physiological conditions in the walls of the arteries is, perhaps, according to KRAWKOW, very closely related to the amyloid substance, but not identical with it, as shown by NEUBERG.[1]

Recently O. HANSSEN has studied the mechanically isolated amyloid obtained from the so-called " sago kernels " of an amyloid spleen, and could not detect any conjugated sulphuric acid in it. According to his investigations true amyloid is not a chondroprotein. MAYEDA[2] has also prepared an amyloid substance free from chondroitin-sulphuric acid. On the other hand, HANSSEN has found that amyloid organs (liver and spleen) are much richer in sulphuric acid that splits off than normal organs, and it is not improbable that the amyloid formation goes hand in hand with the formation of a chondroprotein.

The amyloid prepared by KRAWKOW and NEUBERG had about the same composition: C 49.0–50.1; H 7–7.2; N 14–14.1, and S 1.8–2.8

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[1] Krawkow, Arch. f. exp. Path. u. Pharm., **40**, which contains the literature; Neuberg, Verhandl. d. d. Pathol. Gesellsch., 1904.

[2] Hanssen, Bioch. Zeitschr., **18**; Mayeda, Zeitschr. f. physiol. Chem., **58**.

per cent. The aorta amyloid of man and of the horse contained respectively C 49.6 and 50.5; H 7.2; N 14.4 and 13.8; S 2.3 and 2.5 per cent. As we cannot tell whether the amyloid analyzed was pure or not the results are of questionable value.

According to older investigations amyloid splits, by the action of alkali, into protein and chondroitin-sulphuric acid (see Chapter IX), and according to KRAWKOW it is therefore a firm, perhaps ester-like combination of this acid with protein. The protein, from the investigations of NEUBERG, is of a basic nature and most comparable to the histones. The investigations of MAYEDA do not coincide with this view as the amyloid protein obtained by him did not behave like a histone. Its content of hexone bases was not greater than that of the proteins of the normal organs and this amyloid protein did not yield any histone-peptone. To all appearances, different investigators have worked with different substances and it is possible that in the amyloid degenerated organs partly chondroproteins and partly amyloid proteins may occur, both of which give the color reactions.

Amyloid is an amorphous white substance, insoluble in water, alcohol, ether, dilute hydrochloric and acetic acids. It is soluble in concentrated hydrochloric acid or caustic alkali with decomposition. On boiling with dilute hydrochloric acid it yields sulphuric acid and a reducing substance. It is not dissolved by gastric juice, according to KRAWKOW, which agrees with most of the older reports. It is nevertheless changed so that it is soluble in dilute ammonia, while the typical amyloid is insoluble therein. NEUBERG finds on the contrary that amyloid (from liver) is digested by pepsin as well as by trypsin, although more slowly than fibrin, and that it is also destroyed in autolysis, so that in life an absorption is possible. The amyloid from the "sago" spleen studied by HANSSEN showed the same behavior with gastric juice as KRAWKOW found, while trypsin, as well as autolysis for months, was without action. MAYEDA'S amyloid was gradually dissolved by gastric juice.

Amyloid gives the xanthoproteic reaction and the reactions of MILLON and ADAMKIEWICZ-HOPKINS. Its most important property is its behavior with certain coloring matters. It is colored reddish-brown or a dingy violet by iodine; a violet or blue by iodine and sulphuric acid; red by methylaniline iodide, especially on the addition of acetic acid; and red also by aniline green. Of these color reactions those with aniline dyes are the most important. The iodine reaction appears less constant and is greatly dependent upon the physical condition of the amyloid. The color reactions are due to the presence of the chondroitin-sulphuric acid component, but this stands in opposition to the behavior of the intact amyloid obtained by HANSSEN from the "sago" spleen and the amyloprotein of MAYEDA.

In preparing amyloid, extract the finely divided organs with very dilute ammonia. The undissolved amyloid in the residue, if it does not resist pepsin digestion, can be directly extracted by dilute barium hydrate solution and then precipitated from the filtrate by hydrochloric acid. Otherwise the above mentioned residue is digested for several days with pepsin. The digestion residue is dissolved in dilute ammonia, filtered, the amyloid precipitated by dilute hydrochloric acid, the precipitate dissolved in baryta-water, when the nucleins remain behind, the barium filtrate precipitated with hydrochloric acid and purified, if necessary by repeated solution in ammonia and precipitating with hydrochloric acid, washing and treating with alcohol and ether.

Phosphoglycoproteins. This group includes the phosphorized glycoproteins. They yield no purine bases (nuclein bases) as cleavage products. They are not nucleoproteins and therefore they must not be mistaken for them. On pepsin digestion they may, like certain nucleoalbumins, yield pseudonuclein, but they differ from the nucleoalbumins in that they yield a reducing substance on boiling with dilute acid. They differ from the nucleoproteins, which also yield reducing carbohydrates, in, as above stated, not yielding any purine bases.

Only two phosphorized glycoproteins are known at the present time, namely, *ichthulin*, occurring in carp eggs and studied by WALTER,[1] and which was considered as a vitellin for a time. Ichthulin has the following composition: C 53.52; H 7.71; N 15.64; S 0.41; P 0.43; Fe 0.10 per cent. In regard to solubilities it is similar to a globulin. WALTER has prepared a reducing substance from the pseudonuclein of ichthulin which gave a highly crystalline compound with phenylhydrazine.

Another phosphoglycoprotein is *helicoproteid*, obtained by HAMMARSTEN [2] from the glands of the snail Helix pomatia. It has the following composition: C 46.99; H 6.78; N 6.08; S 0.62; P 0.47 per cent. It is converted into a gummy, levorotatory carbohydrate, called *animal sinistrin*, by the action of alkalies. On boiling with an acid it yields a dextrorotatory reducing substance.

The compound protein found by SHULTZ and DITTHORN [3] in the spawn of the frog probably belongs to this group, but instead of glucosamine it gives galactosamine on cleavage.

B. Nucleoproteins.

By this name we designate those compound proteins which yield protein and nucleic acid on cleavage. The nucleoproteins seem to be widely diffused in the animal body. They occur chiefly in the cell-nuclei, but they also often occur in the protoplasm. They may pass into the animal fluids on the destruction of the cells, hence nucleoproteins have also been found in blood serum and other fluids.

The nucleoproteins may be considered as combinations of a protein with a side chain, which KOSSEL calls the *prosthetic group*. This side chain, which contains the phosphorus, may be split off as nucleic acid on treatment with alkali. The protein may be of different kinds.

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[1] Zeitschr. f. physiol. Chem., **15**.

[2] Hammarsten, Pflüger's Arch., **36**.

[3] Zeitschr. f. physiol. Chem., **29**.

In certain cases this is histone, and the combinations between nucleic acid and protamines are also sometimes classified as nucleoproteins. The combination between protamine and nucleic acid is, it seems, a salt-like combination, and entirely different from the combination of the proteins with nucleic acid in the nucleoproteins. The following facts, given in connection with the nucleoproteins, do not apply to the nucleo-protamines. The nucleoproteins differ not only according to the protein component they contain, but also as to the nucleic acids, which vary among themselves. There are essentially different nucleic acids, some among which contain a pentose carbohydrate while others contain a hexose carbohydrate. The nucleic acids also differ in regard to the amount of purine and pyrimidine bases they contain (see below).

The native nucleoproteins contain a variable, but not a high percentage of phosphorus, which in most of the nucleoproteins investigated, ranges between 0.5 and 1.6 per cent. They also regularly contain iron, and in Octopodes, HENZE[1] has observed an iron-free nucleoprotein with 0.96 per cent copper. The nucleoproteins behave like weak acids, especially those having considerable protein in the molecule. They therefore give the ordinary protein reactions and behave in this regard like the proteins. The nucleoproteins prepared from organs rich in cell nuclei seem to be characterized by containing more phosphorus and having a stronger acid character. All nucleoproteins are bodies that are insoluble in water, but whose alkali combination is soluble in water. From such a solution the nucleoprotein can be precipitated by acetic acid, and in an excess of the acid, the precipitate dissolves with more or less difficulty and in some cases not at all. It dissolves, on the contrary, in very dilute hydrochloric acid. In this respect nucleoproteins are similar to the nucleoalbumins and the mucin substances, but differ from these two groups in that they yield purine bases on hydrolysis. According to PLIMMER and SCOTT[2] the nucleoproteins differ from the nucleo-albumins by the fact that with sodium hydroxide in 1 per cent solution the nucleoalbumins split off phosphoric acid while the nucleoproteins do not. The nucleoproteins give the color reactions of the proteins, but those which have been investigated are dextrorotatory and not lævorotatory (GAMGEE and JONES[3]).

The nucleoproteins are readily modified. The alkali combination soluble in water suffers a decomposition on heating its solution, when as neutral as possible, and coagulated protein separates while a protein rich in phosphorus and poor in protein with strong acid character remains

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[1] Zeitschr. f. physiol. Chem., 55.

[2] Plimmer and Scott, cited in Biochem. Centralbl., 8, p. 109.

[3] Hofmeister's Beiträge, 4.

in solution. By the action of weak acids and by gastric juice a similar cleavage takes place, whereby the protein split off goes into solution while the nucleoprotein rich in phosphorus, so-called *nuclein* (MIESCHER, HOPPE-SEYLER [1]) or *true nuclein*, remains undissolved. As the nuclein is probably nothing but a partly modified nucleoprotein poorer in protein, having a composition varying with the intensity of the cleavage, it seems unnecessary to give the name nuclein thereto. On the other hand, the nucleins have other properties than the nucleoproteins, and as the nucleins bear the same relation to the nucleoproteins that the pseudonuclein does to the nucleoalbumins, we will here give a short description of the nucleins as well as the pseudo- or paranucleins.

Nucleins or true nucleins are formed, as above stated, from nucleoproteins in their peptic digestion or by treatment with dilute acids. It must be remarked that the nucleins are not entirely resistant toward gastric juice, and also that at least one nucleoprotein, namely, the one obtained from the pancreas, completely dissolves, leaving no nuclein residue on treatment with gastric juice (UMBER, MILROY[2]). The nucleins are rich in phosphorus, containing in the neighborhood of 5 per cent. According to LIEBERMANN,[3] metaphosphoric acid can be split off from true nucleins (yeast nuclein). The nucleins are decomposed into protein and nucleic acid by caustic alkali, and as different nucleic acids exist, so also there exist different nucleins. As previously stated proteins may be precipitated in acid solutions by nucleic acids, and in this way, as shown by MILROY, combinations of nucleic acid and proteins may be prepared which behave quite like true nucleins. All nucleins yield purine bases (so-called nuclein bases) on boiling with dilute acids. They act like rather strong acids.

The nucleins are colorless, amorphous and insoluble or only slightly soluble in water. They are insoluble in alcohol and ether. They are more or less readily dissolved by dilute alkalies. The nucleins give the biuret test and MILLON'S reaction. They show a great affinity for many dyes, especially the basic ones, and take these up with avidity from watery or alcoholic solutions. On burning they yield an acid residue which is very difficult to incinerate and which contains metaphosphoric acid. On fusion with saltpeter and soda the nucleins yield alkali phosphates.

To prepare nucleins from cells or tissues, first remove the chief mass of proteins by artificial digestion with pepsin-hydrochloric acid, lixiviate the residue with very dilute ammonia, filter, and precipitate with hydrochloric acid. The precipitate is further digested with gastric juice,

[1] Hoppe-Seyler, Med. chem. Unters., 452.
[2] Umber, Zeitschr. f. klin. Med., **34**; Milroy, Zeitschr. f. physiol. Chem., **22**.
[3] Pflüger's Arch., **47**.

washed and purified by alternately dissolving in very faintly alkaline water and reprecipitating with an acid, washing with water, and treating with alcohol and ether. A nuclein may be prepared more simply by the digestion of a nucleoprotein. In the detection of nucleins we make use of the above-described method, testing for phosphorus in the product after fusing with saltpeter and soda. Naturally the phosphates and phosphatides must first be removed by treatment with acid, alcohol, and ether, respectively. No exact methods are known for the quantitative estimation of nucleins in organs or tissues.

Pseudonucleins or PARANUCLEINS. These bodies are obtained as an insoluble residue on the digestion of certain nucleoalbumins or phosphoglycoproteins with pepsin-hydrochloric acid. Attention is called to the fact that the pseudonuclein may be dissolved by the presence of too much acid or by a too energetic peptic digestion. If the relation between the degree of acidity and the quantity of substance is not properly selected, the formation of pseudonucleins may be entirely overlooked in the digestion of certain nucleoalbumins. Pseudonucleins contain phosphorus, which, as shown by LIEBERMANN,[1] is split off as metaphosphoric acid by mineral acids.

The pseudonucleins are amorphous bodies insoluble in water, alcohol, and ether, but readily soluble in dilute alkalies and barium hydroxide solution. They are readily split by barium hydroxide solution with the splitting off of phosphoric acid, and according to GIERTZ[2] they differ in this regard from the true nucleins, which are neither dissolved nor decomposed by baryta. They are not soluble in very dilute acids, and may be precipitated from their solution in dilute alkalies by adding acid. They give the protein reactions very strongly, but do not yield purine bases.

In preparing a pseudonuclein, dissolve the mother-substance in hydrochloric acid of 1–2 p. m., filter if necessary, add pepsin solution, and allow the mixture to stand at the temperature of the body for about twenty-four hours. The precipitate is filtered off, washed with water, and purified by alternately dissolving in very faintly alkaline water and reprecipitating with acid.

Cleavage Products of the Nucleoproteins.

1. The Nucleic Acids.

All nucleic acids are rich in phosphorus and yield phosphoric acid, purine bases and a carbohydrate or carbohydrate derivative as cleavage products; most of them also contain pyrimidine bases. The older

[1] Ber. d. d. chem. Gesellsch., **21**, and Centralbl. f. d. med. Wissensch., 1889.
[2] Zeitschr. f. physiol. Chem., **28**.

statements as to the occurrence of more than two purine bases in a nucleic acid are not correct and depend upon the fact that the two purine bases xanthine and hypoxanthine can be secondarily formed from guanine and adenine. There is no doubt that the most thoroughly studied nucleic acids, such as the thymus-nucleic acids, the closely related or perhaps identical acids of the salmon sperm (salmo-nucleic acid), of the herring sperm and burbot sperm, and of the pancreas, do not contain more than two purine bases, namely, guanine and adenine.

Of the known nucleic acids we have two, the guanylic acid and inosinic acid, which contain only one purine base, namely, guanine and hypoxanthine, respectively. These two acids do not contain any pyrimidine bases, which are found thus far in all carefully investigated nucleic acids. The occurrence of pyrimidine bases is somewhat different in the various nucleic acids. In one group of animal nucleic acids (thymonucleic acids) thymine, cytosine and uracil are found, the uracil being produced secondarily from the cytosine. The plant nucleic acids (the triticonucleic acid and the yeast nucleic acid, which may perhaps be identical with it) do not contain any thymine and yields as nitrogenous cleavage products besides the two purine bases only cytosine and uracil.

All nucleic acids, as above stated, contain a carbohydrate group. In the plant nucleic acids and in two animal ones, the guanylic and inosinic acids, the carbohydrate is a pentose. In the remaining animal nucleic acids it is on the contrary a hexose or at least a hexacarbohydrate.

The nature of this hexacarbohydrate has not been determined and the nature of the pentoses occurring in the nucleic acids is also a disputed point. Based upon the investigations of NEUBERG we have considered the pentose of guanylic acid and of inosinic acid as *l*-xylose. The correctness of this view is disputed by others. According to LEVENE and JACOBS the pentose of all nucleic acids containing pentose, is *d*-ribose. HAISER and WENZEL who for a time considered the pentose of inosinic acid as *d*-xylose are now of the view that it is probably *d*-ribose. The view of LEVENE and JACOBS, that the pentose of the guanylic acid is *d*-ribose has received important support by the investigations of SCHULZE and TRIER on the identity of the plant guaninpentoside *vernine* with the guaninpentoside (see below) prepared by LEVENE and JACOBS. Still we have no explanation why NEUBERG and REWALD[1] obtained only *l*-xylose from the pancreas on the hydrolysis of the entire organ, and LEVENE and JACOBS on the contrary only *d*-ribose.

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[1] Neuberg and Brahn, Bioch. Zeitschr., **5**; see also Ber. d. d. chem. Gesellsch **41** and **42**; LEVENE and JACOBS, Ber. d. d. chem. Gesellsch., **42** and **43**; Haiser and Wenzel, Monatsh. f. Chem., **31**; Schulze and Trier, Zeitschr. f. physiol. Chem., **70**; Rewald, Ber. d. d. chem. Gesellsch., **42**.

All nucleic acids contain phosphoric acid. The relation between phosphorus and nitrogen is as 1:4 in the inosinic acid and as 1:5 in the guanylic acid. In the thymus- and the salmo-nucleic acids the relation according to SCHMIEDEBERG is 4:14 and according to STEUDEL 4:15. In the triticonucleic acid, OSBORNE and HARRIS found the relation 4:16; in the yeast nucleic acid, LEVENE and JACOBS found it was equal to 4:15.

According to the number of bases contained in the nucleic acids we can differentiate between the simple nucleic acid with only one base and the complex nucleic acids with several bases. LEVENE and MANDEL [1] have called the first (inosinic acid, guanylic acid) *nucleotides* or *mononucleotides* and the last *polynucleotides*.

The properties and the constitution of the nucleic acids, as far as we know them, have been determined essentially by the work of KOSSEL and his pupils, by SCHMIEDEBERG, STEUDEL and LEVENE [2] and their collaborators.

On complete acid hydrolysis the nucleic acids are split into the three above mentioned components, phosphoric acid, carbohydrate and bases. The purine bases are more readily split off than the pyrimidine bases and on careful acid hydrolysis of thymus nucleic acid, a new acid, the *thyminic acid* of STEUDEL and BRIGL is obtained. This acid is very similar to the thyminic acid of KOSSEL and NEUMANN [3] with the barium salt, $C_{16}H_{23}N_3P_2O_{12}Ba$, and the *nucleotinphosphoric acid* of SCHMIEDEBERG. This acid differs probably from the original nucleic acid only by the absence of purine bases. By the action of strong nitric acid in the cold we can, according to the method suggested by STEUDEL, split off the purine bases while nearly all the phosphoric acid remains in organic combination with the carbohydrate complexes.

The hydrolyses of pentose containing nucleic acids as carried out by LEVENE and JACOBS in neutral, or, if the pyrimidine complexes of the plant nucleic acid were being studied, in ammoniacal reaction, by heating to high temperatures in the autoclave or in sealed tubes, are of special interest. In these cases the binding with the phosphoric acid was rup-

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[1] Ber. d. d. chem. Gesellsch., **41**.

[2] The work of Kossel and his pupils on the nucleic acids can be found in: Arch. f. (Anat. u.) Physiol. 1892, 1893 and 1894; Sitz. Ber. d. Berl. Akad. d. Wiss., 18, 1894; Centralbl. f. d. med. Wiss. 1893; Ber. d. d. chem. Gesellsch., **26** and **27**; Zeitschr. f. physiol. Chem., **23** and **38**; see also Neumann, Arch. f. (Anat. u.) Physiol., 1898 and 1899 Suppl.; Miescher, Hoppe-Seyler's Med. chem. Unters., p. 441 and Arch. f. exp. Path. u. Pharm., **37**; Schmiedeberg, *ibid.*, **37**, **43**, and **57**; Altman, Arch. f. (Anat. u.) Physiol., 1889; Steudel, *ibid.*, **42**, **43**, **46**, **49**, **50**, **52**, **53**, **55**, **56**, **70**, **77**; Ascoli, Zeitschr. f. physiol. Chem., **28** and **31**; Levene, *ibid.*, **32**, **37**, **38**, **39**, **43**, **45**; Levene and Mandel, *ibid.* **46**, **47**, **49**, **50**; Inouye and Kotake, *ibid.*, **46**; Levene and Jacobs, Ber. d. d. chem. Gesellsch., **42**, **43**, **44**; with La Forge, *ibid.*, **43** and **45**.

[3] Steudel and Brigl, Zeitschr. f. physiol. Chem., **70**; Kossel and Neumann, *ibid.*, **22**.

tured while the binding between the pentose and purine bases remained intact. In this manner they obtained *pentosides*, i.e., glucoside-like combination between pentose and a purine base. These pentosides have also been called *nucleosides* and such a nucleoside was the *inosine*, which was first found by HAISER and WENZEL [1] and which is the pentoside of inosinic acid and is a combination of hypoxanthine with pentose (*d*-ribose). The other three nucleosides *adenosine*, *guanosine* and *xanthosine* have been prepared by LEVENE and JACOBS.

The nucleosides are crystalline bodies which give crystalline combinations. Of special interest is guanosine because it is identical with the base vernine, occurring in the plant kingdom and discovered by SCHULZE [2] and because of the identity of the pentose occurring in both has been positively proved. The guanosine has also been found by LEVENE and JACOBS [3] in the pancreas. On acid hydrolysis every nucleoside splits into purine base and pentose. By the action of nitrite and glacial acetic acid the guanosine is transformed into xanthosine and the adenosine into inosine.

MANDEL and DUNHAM have prepared, from acetone-yeast, a crystalline adenine-hexose compound corresponding to the pentoside but whose relation to the cleavage products of nucleic acids is not known. From thymus nucleic acid LEVENE and JACOBS [4] have later isolated a guanine hexoside.

The pyrimidine complexes corresponding to the nucleosides also contain (in the plant nucleic acids) pentose, according to LEVENE and LA FORGE [5] but in much firmer bondage. This is the reason why they give only a faint orcin reaction, are much more resistant to enzymes than the purine nucleosides and give off furfurol only very slowly on distilling with hydrochloric acid. Still they contain pentose and pyrimidine bases in equimolecular proportions. The pyrimidine complexes are called *cytidine* and *uridine*, the first containing cytosine and the second uracil. Uridine is crystalline; the cytidine has not been obtained in a crystalline form but it gives several crystalline salts. The uridine is claimed to exist pre-formed in the yeast nucleic acid and not produced secondarily from the cytidine.

Based upon the investigations carried out by STEUDEL, LEVENE and JACOBS we can for the present represent the structure of the nucleic acids in the following way:

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[1] Monatsh. f. Chem., **29**.

[2] E. Schulze and Bosshard, Zeitschr. f. physiol. Chem., **10**; with Trier, *ibid.*, **70**.

[3] Bioch. Zeitschr., **28**.

[4] Mandel and Dunham, Journ. of biol. Chem., **11**; Levene and Jacobs, *ibid.*, **12**.

[5] Ber. d. d. chem. Gesellsch., **45**.

The **simple nucleic acids** are ester-like combinations between phosphoric acid and a purine base-pentoside.

The **complex nuclei acids** are complex molecules each composed of four simple nucleic acids (nucleotides). In regard to the complex nucleic acids we differentiate between two groups.

The acids of the *thymonucleic acid group* are, according to STEUDEL, tetrabasic phosphoric acid ester which corresponding to each phosphorus atom, contains a hexose group and one of the four bases, guanine, adenine, cystosine and thymine. From the name of this group we infer that these acids contain thymine.

The *plant nucleic acid group* differs from the preceding by the following. They do not contain any thymine but uracil instead. They do not contain any hexose but do contain pentose. In the acids of this group for each atom of phosphor we have 1 mol. pentose and on each the purine and pyrimidine bases are combined.

It must be remarked that the complex nucleic acids have not been prepared from isolated component proteins but generally from organs, namely perhaps from a mixture of different nucleoproteins and that for this reason we do not know whether these acids are chemical individuals or only a mixture of closely related simple nucleic acids. On the other hand it is also possible that the simple nucleic acids originate from more complex nucleic acid by cleavage because such cleavages are in fact known. Such an assumption does not apply at least for the guanylic acid from the pancreas as it is obtained from a compound protein with only one base, namely guanine.

All nucleic acids are amorphous, white, and have an acid reaction. They are readily soluble in ammoniacal or alkaline water. They also dissolve in concentrated acetic acid and form insoluble salts with copper chloride and salts of the heavy metals, and as a rule insoluble basic salts with the alkaline earths. Their solubility in water is very different. Inosinic acid, for example, is very readily soluble in cold water while β-guanylic acid is soluble with difficulty. The complex nucleic acids are also soluble with difficulty in cold water. The solution of their alkali combination is not as a rule precipitated by acetic acid but is precipitated by a slight excess of hydrochloric acid, especially in the presence of alcohol. The nucleic acids soluble in dilute acids give in such solution a precipitate with proteins, which are considered as nucleins. All nucleic acids are insoluble in alcohol and ether. They do not give either the biuret test or MILLON'S reaction. The nucleic acids are optically active and, with the exception of inosinic acid (GAMGEE and JONES) and of guanylic acid (LEVENE and JACOBS [1]), are dextro-rotatory.

[1] Gamgee and Jones, Proc. Roy. Soc., **72**; Levene and Jacobs, Journ. of biol. Chem., **12**.

The proteolytic enzymes, such as pepsin and trypsin, decompose the nucleoproteins more or less; the nucleic acids are apparently not split by these enzymes or at least not as far as phosphoric acid and purine bases. Such a cleavage can, on the contrary, be brought about by erepsin (NAKAYAMA) or by other closely allied enzymes found in various organs which have been called *nucleases*. Micro-organisms can also bring about a more or less deep cleavage of the nucleic acids (SCHITTENHELM and SCHRÖTER [1]).

LEVENE and MEDIGRECEANU [2] differentiate between three kinds of nucleases namely, nucleinases, nucleotidases and nucleosidases. The *nucleinases*, which are found in the pancreatic juice and all organs investigated, but not in gastric juice, acts only upon the complex nucleic acids and splits them into nucleotides. The *nucleotidases*, which, with the exception of the gastric and pancreatic juices, occurs all over and especially in the intestinal mucosa, split the simple nucleic acids (mononucleotides) into phosphoric acid and the corresponding nucleoside (purine pentoside). The *nucleosidases*, which are not found in the gastric, pancreatic or intestinal juices, nor in the blood or the pancreas but in other organs, split the nucleosides into purine base and pentose. It is unknown how the cleavage of the pyrimidine and hexose complexes of the nucleic acids is brought about.

According to W. JONES [3] the purine bases of the nucleic acids can be deamidized without being previously split off as free base from the acid. Thus the pig-pancreas contains an adenosin-deamidase which deamidizes the still combined adenine. On the contrary the same organ also contains a guanase which deamidizes the free guanine but does not contain a guanosine deamidase. The pig liver, in which only traces of guanase occur, contain on the contrary a guanosine-deamidase. Recent investigations of SCHITTENHELM and K. WIENER [4] show that we must also admit of nucleoside-deamidases besides purine deamidases.

Inosinic Acid, $C_{10}H_{13}N_4PO_8$ was first isolated by LIEBIG from the flesh of certain animals and then closely studied by HAISER. It is obtained from beef extracts, and according to the investigations of NEUBERG and BRAHN, FR. BAUER, and LEVENE and JACOBS it is a simple nucleic acid.[5]

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[1] Nakayama, Zeitschr. f. physiol. Chem., **41**; Iwanoff, *ibid.*, **39**; Fr. Sachs, "Ist die Nuklease mit dem Trypsin identisch?" Inaug.-Dissert, Heidelberg, 1905; Schittenhelm and Schröter, f. physiol. Zeitschr. Chem., **41**.

[2] Journ. of biol. Chem., **9**.

[3] Journ. of biol. Chem., **9**.

[4] Zeitschr. f. physiol. Chem., **77**.

[5] Liebig, Annal. d. chem. u. Pharm., **62**; Haiser, Monatsh. f. chem., **16**; Neuberg and Brahn, Biochem. Zeitschr., **5** and Ber. d. d. chem. Gesellsch., **41**, p. 3376; Bauer Hofmeister's Beiträge, **10**; Levene and Jacobs, Ber. d. d. chem. Gesellsch., **41**, p. 2703.

On hydrolysis it yields phosphoric acid, hypoxanthine and pentose, according to the equation:

$$C_{10}H_{13}N_4PO_8+2H_2O=H_3PO_4+C_5H_4N_4O+C_5H_{10}O_5.$$

The pentose, whose somewhat disputed nature has been discussed on page 178, is combined with hypoxanthine in a glucoside-like combination forming the pentoside inosine, which, according to LEVENE and JACOBS, is combined with the phosphoric acid, like an ester by means of the δ-carbon atom of the pentose (ribose).

Inosinic acid is amorphous, syrupy, readily soluble in water and precipitable by alcohol. It is lævo-rotatory; for the Ba salt containing hydrochloric acid NEUBERG and BRAHN found $(a)_D=-18.5°$ at 16° C. It gives several crystalline salts among which the barium salt, which is soluble with difficulty in water, must be mentioned.

In regard to the preparation of this acid we must refer to the works of HAISER, NEUBERG and BRAHN, LEVENE and JACOBS mentioned in footnote 5, page 182.

Guanylic acid. This acid, which was first prepared by BANG from the pancreas has also been found by JONES and ROWNTREE in the spleen and by LEVENE and MANDEL[1] in the liver. As cleavage products it yields guanine, pentose and phosphoric acid and therefore its simplest formula is $C_{10}H_{14}N_5PO_8$. This formula is accepted also by STEUDEL and BRIGL and by LEVENE and JACOBS, while BANG basing his views on the results of elementary analysis gives the formula $C_{44}H_{66}N_{20}P_4O_{34}$. According to this formula the acid would contain besides, guanine, pentose and phosphoric acid also an unknown residue, $C_4H_{10}O_2$, and according to BANG is not a simple nucleic acid but would occupy an intermediary position between the inosinic acid and the thymus nucleic acid. In opposition to this it must be remarked that LEVENE and JACOBS[2] have recently prepared the crystalline brucine salt of the acid and the analysis of this salt as well as the barium salt substantiates the first mentioned, simple formula. In regard to the pentose of guanylic acid see page 178.

The acid first described by BANG, the β-acid, is soluble with great difficulty in cold water and rather readily soluble in boiling water. It is easily precipitated by acetic acid from the solution of the alkali combination in water. The β-acid may, according to BANG, be derived from another guanylic acid, the α-guanylic acid, by the action of the alkali. The

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[1] Bang, Zeitschr. f. physiol. Chem., **26**; with Raaschou, Hofmeister's Beiträge, **4**; Jones and Rowntree, Journ. of biol. chem., **4**; Levene and Mandel, Biochem. Zeitschr. **10**.

[2] Steudel and Brigl, Zeitschr. f. physiol. Chem., **68**; Bang, *ibid.*, **69** and Bioch. Zeitschr., **26**; Levene and Jacobs, Journ. of biol. Chem., **12**.

α-guanylic acid is readily soluble, even in cold water, and it is also similar to thymus nucleic acid in other respects. It is precipitated from the solution of its salts by hydrochloric acid but not by acetic acid, and its solutions precipitate proteins. STEUDEL and BRIGL believe that the β-acid is a potassium salt and that the α-acid is the actual acid, but this view BANG disputes. LEVENE and JACOBS found that the acid contaminated with alkali does not gelatinize while the pure acid does. The specific rotation of the latter was $(a)_D = -1.27°$ at 25° C.

In regard to the preparation of guanylic acid we refer, to the work of BANG, LEVENE and JACOBS.[1]

Thymonucleic Acids. A. NEUMANN has isolated two nucleic acids, α- and β-thymus nucleic acid, from the thymus gland. The α-acid is soluble with difficulty, and in proper concentration gives a sodium salt which gelatinizes in proper concentration, and a barium salt which is precipitated by barium acetate in substance (KOSTYTSCHEW). The barium salt of the β-acid is not precipitated by barium acetate. The α-acid is designated as anhydric by SCHMIEDEBERG,[2] and the β-acid as hydrate, and the first can be transformed into the second by heating. This transformation, according to KOSTYTSCHEW, is a decomposition whereby two-thirds of the purine bases are split off.

According to SCHMIEDEBERG the thymus nucleic acid is identical with the salmo-nucleic acid (from salmon sperm), and also according to STEUDEL probably with the acid from the herring sperm. Other nucleic acids, at least those very closely related to this nucleic acid, have been prepared from the sperm of the burbot (Lota vulgaris) by ALSBERG, of the sturgeon (NOLL) and of the sea-urchin (MATHEWS), also from ox-sperm, brain, spleen (LEVENE), pancreas (LEVENE, v. FÜRTH and JERUSALEM, STEUDEL), mammary glands and kidneys (LEVENE and MANDEL[3]) and from other organs.

At the present time we are not agreed as to the formula for the most carefully studied thymonucleic acids (from the thymus, herring and salmon sperms). According to the numerous analyses of SCHMIEDEBERG and his collaborators for every 4 atoms of phosphorus there occur 14 atoms of nitrogen. The relationship of C to P was 40 to 4 and the relationship of C to N in 12 out of 15 preparations was 40 to 14, and only in 3 preparations 40 to 15. From these facts SCHMIEDEBERG has given the acid

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[1] See footnotes 1 and 2, p. 183.

[2] A. Neumann, Arch. f. (Anat. u.) physiol, 1898 and 1899; Kostytschew, Zeitschr. f. physiol. Chem., **39**; Schmiedeberg, l. c.

[3] Alsberg, Arch. f. exp. Path. u. Pharm., **51**; Noll, Zeitschr. f. physiol. Chem., **25**; Mathews, *ibid.*, **23**; v. Fürth and Jerusalem Hofmeister's Birträge, **10** and **11**; Steudel, Zeitschr. f. physiol. Chem., **53**; Levene and Mandel, *ibid.*, **46**, **47**. See also footnote 2, p. 179.

the formula $C_{40}H_{56}N_{14}O_{16}.2P_2O_5$. According to STEUDEL for every 4 atoms of phosphorus we have 15 atoms nitrogen and from this he has calculated the formula $C_{43}H_{61}N_{15}P_4O_{34}+9H_2O$ for the acid containing water.

The probable constitution of the thymo-nucleic acids has been previously indicated and as positively known cleavage products we have at least phosphoric acid, a hexose carbohydrate, guanine, adenine, thymine and cytosine.

The thymo-nucleic acids have the reactions as given for the complex nucleic acids. They are amorphous, dextro-rotatory, and soluble in cold water with difficulty. They form soluble salts with alkalies and the acid is precipitated from these solutions by mineral acid but not by acetic acid. Tannic acid alone does not cause a precipitate but does in the presence of sodium acetate. Proteins precipitate their solutions containing acetic acid. The two special thymo-nucleic acids differ from each other by the different behavior of their salts (see above).

The preparation of the nucleic acids is based in the first place always upon the cleavage of the nucleoprotein into protein and nucleic acid by the action of alkali and then separating the nucleic acids from the protein. The operations necessary for purifying the nucleic acids from proteins are very complicated and we must refer to the works of SCHMIEDEBERG, NEUMANN, LEVENE, and others.[1]

Plant Nucleic Acids. The two best known acids of this group are the *yeast nucleic acid* and the *triticonucleic acid* isolated from the wheat embryo. The identity of these two acids, as suggested by OSBORNE and HARRIS has become more and more probable. According to KOWALEWSKY[2] the yeast nucleic acid contain only adenine, guanine and cytosine, the uracil is only formed secondarily from the cytosine. The yeast nucleic acid may perhaps be a triphosphoric acid with three molecules of pentose each with a molecule of adenine, guanine and cytosine.

This view stands in opposition to the observations of LEVENE and JACOBS[3] that the yeast nucleic acid contains one molecule of pentose combined with adenine and guanine, and besides this it contains two pyrimidinehexose complexes, cytidine and uridine.

The triticonucleic acid yields also, as OSBORNE and HEYL, WHEELER and JOHNSON and recently LEVENE and LA FORGE[4] have shown, the same hydrolytic products as the yeast nucleic acid and both contain *d*-ribose.

[1] Schmiedeberg, Arch. f. exp. Path. u. Pharm., **43** and **57**; Herlant, *ibid.*, **44**; Neumann, Arch. f. (Anat. u.) Physiol. 1899 Supplb.; Levene, Zeitschr. f. physiol. Chem., **32** and **45**; Kostytschew, l. c.

[2] Osborne and Harris, Zeitschr. f. physiol. Chem., **36**; Kowalewsky, *ibid.*, 69.

[3] Ber. d. d. chem. Gesellsch., **44**.

[4] Osborne and Heyl, Amer. Journ. of Physiol., **21**; Wheeler and Johnson, Amer. Chem. Journ., **29**; Levene and La Forge, Ber. d. d. Chem., Gesellsch., **43**.

The somewhat different results found on the elementary analysis of these two acids do not seem to be of very great importance and we have strong evidence for the identity of these acids. OSBORNE and his collaborators found the formula $C_{41}H_{61}N_{16}P_4O_{31}$ for triticonucleic acid.

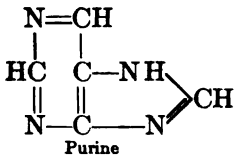
The plant nucleic acids have the general reactions of the complex nucleic acids but can be precipitated by an excess of acetic acid. They are dextro-rotatory.

In regard to their preparation we refer to the works of KOSSEL, OSBORNE and HARRIS and to LEVENE and co-workers.[1]

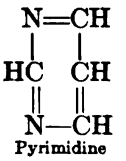
Plasminic acid is an acid which was prepared by ASCOLI and KOSSEL [2] by the action of alkali upon yeast. It contains iron, and is soluble in very dilute hydrochloric acid (1 p. m.). It is still a question whether it is a mixture or a chemical individual.

2. Purine Bases.

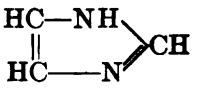
The cleavage products obtained from the nucleic acids, the *nuclein bases*, which are also called *alloxuric bases* by KOSSEL and KRÜGER, are members of the larger group of *purines*, to which also belongs the uric acid which is a substance occurring in the animal body. The constitution of these bodies has been explained by E. FISCHER,[3] and he has prepared many of the bodies synthetically. They can all be derived from the synthetically prepared purine, $C_5H_4N_4$, which has the formula given below and which may be considered as a combination of a pyrimidine ring with an imidazole ring.



Purine

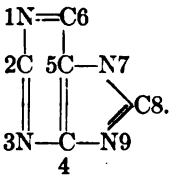


Pyrimidine



Imidazole

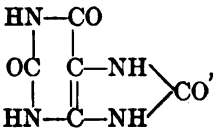
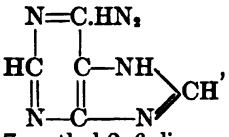
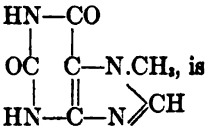
The different purine bodies are derived therefrom by the substitution of the various hydrogen atoms by hydroxyl, amide, or alkyl groups. In order to signify the different positions of substitution FISCHER has proposed to number the nine members of the purine nucleus in the following way:



[1] See footnote 2, p. 179, and footnote 3 and 4, p. 185.

[2] Ascoli, Zeitschr. f. physiol. Chem., 28.

[3] See E. Fischer, Untersuchungen in der Puringruppe (1882–1906) Berlin, 1907.

For example, uric acid,  , is 2, 6, 8-trioxypurine; adenine,  , is 6-aminopurine, and heteroxanthine,  , is 7-methyl-2, 6-dioxypurine, etc.

The starting-point used by FISCHER for the synthetical preparation of the purine bases was 2, 6, 8-trichlorpurine, which is obtained, with 8-oxy-2, 6-dichlorpurine as an intermediary product, from potassium urate and phosphorus oxychloride.

The purine bodies or alloxuric bodies, found in the animal body or its excreta are as follows: *Uric acid, xanthine, heteroxanthine, 1-methylxanthine, paraxanthine, guanine, epiguanine, hypoxanthine, episarkine, adenine.* The bodies *theobromine, theophylline,* and *caffeine,* occurring in the vegetable kingdom, stand in close relation to this group.

The composition of the purine bodies most important from a physiological standpoint is as follows:

Uric acid,	$C_5H_4N_4O_3$	2, 6, 8-trioxypurine
Xanthine	$C_5H_4N_4O_2$	2, 6-dioxypurine
1-methylxanthine,	$C_6H_6N_4O_2$	1-methyl " "
Heteroxanthine,	$C_6H_6N_4O_2$	7 " " "
Theophylline,	$C_7H_8N_4O_2$	1, 3-dimethyl " "
Paraxanthine,	$C_7H_8N_4O_2$	1, 7- " " "
Theobromine	$C_7H_8N_4O_2$	3, 7- " " "
Caffeine,	$C_8H_{10}N_4O_2$	1, 3, 7-trimethyl " "
Hypoxanthine,	$C_5H_4N_4O$	6-oxypurine
Guanine	$C_5H_5N_5O$	2-amino " "
Epiguanine,	$C_6H_7N_5O$	7-methyl " " " "
Adenine	$C_5H_5N_5$	6-aminopurine
Episarkine,	$C_6H_8N_2O$(?)	

After SALOMON[1] had shown the occurrence of xanthine bodies in young cells, the importance of the purine bases as decomposition products of cell nuclei and of nucleins was shown by the pioneering researches of KOSSEL, who discovered adenine and theophylline. In those tissues in which, as in the glands, the cells have kept their original state, the purine bases are not found free, but in combination with other atomic groups (nucleic acids). In such tissues, on the contrary, as in muscles, which are poor in cell nuclei, the purine bases are found in the free state. Since the purine bases, as suggested by KOSSEL, stand in close relationship to the cell nucleus, it is easy to understand why the quantity of these bodies is so greatly increased when large quantities of nucleated

[1] Sitzungsber. d. Bot. Verein der Provinz Brandenburg, 1880.

cells appear in such places as were before endowed in a relatively poor manner. As an example of this, the blood, in leucæmia, is extremely rich in leucocytes. In such blood KOSSEL [1] found 1.04 p. m. purine bases, against only traces in the normal blood. That these bases are also intermediate steps in the formation of uric acid in the animal organism is probable, and will be shown later (see Chapter XIV).

Only a few of the purine bases have been found in the urine or in the muscles. Only four bases—xanthine, guanine, hypoxanthine, and adenine—have been obtained, thus far, as cleavage products of nucleins, and these do not always have a primary origin. In regard to the purine bodies from other substances we refer the reader to their respective chapters. Only the above four bodies, the real nuclein bases, will be considered at this time.

Of these four bodies, xanthine and guanine form one special group and hypoxanthine and adenine another. By the action of nitrous acid guanine is converted into xanthine and adenine into hypoxanthine.

$$\underset{\text{Guanine}}{C_5H_4N_4O.NH} + HNO_2 = \underset{\text{Xanthine}}{C_5H_4N_4O_2} + N_2 + H_2O;$$

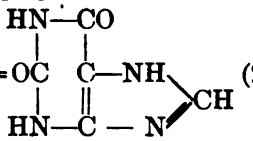
$$\underset{\text{Adenine}}{C_5H_4N_4.NH} + HNO_2 + \underset{\text{Hypoxanthine}}{C_5H_4N_4O} + N_2 + H_2O.$$

Similar transformation, where xanthine and hypoxanthine are produced secondarily, may also occur in the hydrolysis of nucleic acids as well as in putrefaction and by the action of special enzymes. The researches of SCHITTENHELM, LEVENE, JONES, PARTRIDGE, WINTERNITZ, and BURIAN have shown that in various organs deamination enzymes, such as *guanase* and *adenase*, occur, which convert guanine and adenine into xanthine and hypoxanthine respectively, and also *oxidases* which oxidize hypoxanthine into xanthine and this then into uric acid. This formation of uric acid from the purine bases, which will be discussed in detail in a following chapter (XIV), is of very great interest.

The nuclein bases form crystalline salts with mineral acids, which, with the exception of the adenine salts, are decomposed by water. They are easily dissolved by alkalies, while with ammonia their action is somewhat different. They are all precipitated from acid solution by phosphotungstic acid; they also separate as silver compounds on addition of ammonia and ammoniacal silver-nitrate solution. These precipitates are soluble in boiling nitric acid of 1.1 specific gravity. All purine bodies are also precipitated by FEHLING'S solution (see Chapter III) in the presence of a reducing substance such as hydroxylamine (DRECHSEL and BALKE). Copper sulphate and sodium bisulphite may also be used to

[1] Zeitschr. f. physiol. Chem., 7.

advantage in their precipitation (KRÜGER)[1]. This behavior of the purine bases serves just as well as the behavior with the silver solution for their precipitation and preparation.

Xanthine, $C_5H_4N_4O_2$,  (2, 6-dioxypurine), is found

in several cellular organs. It occurs in small quantities as a physiological constituent of urine, and it occasionally has been found as a urinary sediment, or calculus. It was first observed in such a stone by MARCET. Xanthine is found in larger amounts in a few varieties of guano (Jarvis guano).

Xanthine can be prepared, according to E. FISCHER, by boiling uric acid with 25 per cent hydrochloric acid or, according to SUNDVIK,[2] by heating uric acid with anhydrous oxalic acid in glycerin to about 200° C.

Xanthine is amorphous, or forms granular masses of crystals, or may also, according to HORBACZEWSKI,[3] separate as masses of shining, thin, large rhombic plates with 1 mol. water of crystallization. It is very slightly soluble in water, in 14,151–14,600 parts at 16° C., and in 1300–1500 parts at 100° C. (ALMÉN[4]). It is insoluble in alcohol or ether, but is readily dissolved by alkalies and with difficulty by dilute acids. With hydrochloric acid it gives a crystalline, difficultly soluble combination. With very little caustic soda it gives a readily crystallizable compound, which is easily dissolved by an excess of alkali. Xanthine dissolved in ammonia gives with silver nitrate an insoluble, gelatinous precipitate of silver xanthine. This precipitate is dissolved by hot nitric acid, and by this means an easily soluble crystalline double compound is formed. Xanthine in aqueous solution is precipitated on boiling with copper acetate. At ordinary temperatures xanthine is precipitated by mercuric chloride and by ammoniacal basic lead acetate. It is not precipitated by basic lead acetate alone.

When evaporated to dryness in a porcelain dish with nitric acid, xanthine gives a yellow residue, which turns, on the addition of caustic soda, first red, and after heating, purple-red. If we place some chlorinated lime with some caustic soda in a porcelain dish and add the xanthine

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[1] Balke, Zur Kenntnis der Xanthinkörper, Inaug.-Diss. Leipzig, 1893; Krüger Zeitschr. f. physiol. Chem., 18.

[2] E. Fischer, Ber. d. d. chem. Gesellsch, 43; Sundvik, Zeitschr. f. physiol. Chem. 76. In regard to the synthesis of xanthine and other purines see E. Fischer, footnote 3, p. 186.

[3] Zeitschr. f. physiol. Chem., 23.

[4] Journ. f. prakt. Chem., 96.

to this mixture, at first a dark green and then quickly a brownish halo forms around the xanthine grains and finally disappears (HOPPE-SEYLER). If xanthine is warmed in a small vessel on the water-bath with chlorine-water and a trace of nitric acid, and evaporated to dryness, and the residue is then exposed under a bell-jar to the vapors of ammonia, a red or purple-violet color is produced (WEIDEL'S reaction). E. FISCHER[1] has modified WEIDEL'S reaction in the following way: He boils the xanthine in a test-tube with chlorine-water or with hydrochloric acid and a little potassium chlorate, then evaporates the liquid carefully, and moistens the dry residue with ammonia.

Guanine, $C_5H_5N_5O$, $= H_2N.C\begin{matrix} \| \\ N \end{matrix}\!\!-\!\!\begin{matrix} C \\ | \end{matrix}\!\!-\!\!\begin{matrix} CO \\ | \end{matrix}$ $\begin{matrix} \\ C-NH \\ \| \quad \diagdown \\ N-C- \quad N \end{matrix}\!\!>\!\!CH$ (2-amino-6-oxypurine).

Guanine is found in all organs rich in cells. It is further found in the muscles (in very small amounts), in the scales and in the air-bladder of certain fishes, as iridescent crystals of guanine-lime; in the retinal epithelium of fishes, in guano, and in the excrement of spiders it is found as chief constituent. It also occurs in human and pig urine. Under pathological conditions it has been found in leucæmic blood, and in the muscles, ligaments, and articulations of pigs with guanine-gout.

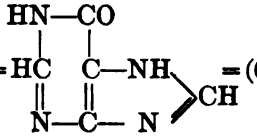
Guanine is a colorless, ordinarily amorphous powder, which may be obtained as small crystals by allowing its solution, in concentrated ammonia, to evaporate spontaneously. According to HORBACZEWSKI it may under certain conditions appear in crystals similar to creatinine-zinc chloride. It is insoluble in water, alcohol, and ether. It is rather easily dissolved by mineral acids and readily by alkalies, but it dissolves with great difficulty in ammonia. According to WULFF[2] 100 cc. of cold ammonia solution containing 1, 3, or 5 per cent NH_3 dissolve 9, 15, or 19 milligrams of guanine respectively. The solubility is relatively increased in hot ammonia solution. The hydrochloride readily crystallizes, and has been recommended by KOSSEL[3] for the microscopical detection of guanine, on account of its behavior toward polarized light. The sulphate contains 2 molecules of water of crystallization, which is completely expelled on heating to 120° C., and this fact, as well as the fact that guanine yields guanidine on decomposition with chlorine-water, differentiates it from 6-amino-2-oxypurine, which is considered as an oxidation product of adenine and possibly occurs as a chemical metabolic

[1] Ber d deutsch. chem. Gesellsch., **30**, 2236.

[2] Zeitschr f. physiol. Chem., **17**.

[3] Ueber die chem. Zusammensetz. der Zelle, Verh. d. physiol. Gesellsch. zu Berlin 1890-91, Nos. 5 and 6.

product (E. FISCHER). The 6-amino-2-oxypurine sulphate contains only 1 molecule of water of crystallization, which is not expelled at 120° C. Very dilute guanine solutions are precipitated by both picric acid and metaphosphoric acid. These precipitates may be used in the quantitative estimation of guanine. The silver compound dissolves with difficulty in boiling nitric acid, and on cooling the double compound crystallizes out readily. Guanine acts like xanthine in the nitric-acid test, but gives with alkalies on heating a more bluish-violet color. A warm solution of guanine hydrochloride gives with a cold saturated solution of picric acid a yellow precipitate consisting of silky needles (CAPRANICA). With a concentrated solution of potassium bichromate a guanine solution gives a crystalline, orange-red precipitate, and with a concentrated solution of potassium ferricyanide a yellowish-brown, crystalline precipitate (CAPRANICA). It also gives a compound with picrolonic acid (LEVENE [1]). Guanine gives WEIDEL'S reaction.

Hypoxanthine, SARKINE, $C_5H_4N_4O$,  =(6-oxypurine).

This body has been found in all cellular organs and in meat extracts, and as a cleavage product of inosinic acid. It is especially abundant in the sperm of the salmon and carp. Hypoxanthine occurs also in the marrow and in very small quantities in normal urine, and, as it seems, also in milk. It is found in rather considerable quantities in the blood and urine in leucæmia.

Hypoxanthine can be obtained according to SUNDVIK'S [2] method from uric acid or xanthine by heating with a formate or more simply by heating with chloroform in alkaline solution.

Hypoxanthine forms very small, colorless, crystalline needles. It dissolves with difficulty in cold water, but the claims concerning solubility therein are very contradictory.[3] It dissolves more readily in boiling water, in about 70–80 parts. It is almost insoluble in alcohol, but is dissolved by acids and alkalies. The compound with hydrochloric acid is crystalline, and is more soluble than the corresponding xanthine derivative. It is easily soluble in ammonia. The silver compound dissolves with difficulty in boiling nitric acid. On cooling, a mixture of two hypoxanthine silver-nitrate compounds possessing an inconstant composition separates out. On treating this mixture with ammonia and an excess of silver nitrate and heating, a silver hypoxanthine is

[1] Capranica, Zeitschr. f. physiol. Chem., 4; Levene, Bioch. Zeitschr., 4.
[2] l. c. and Skand, Arch. f. Physiol., 25.
[3] See E. Fischer, Ber. d. deutsch. chem. Gesellsch., 30.

formed, which when dried at 120° C. has a constant composition, $2(C_5H_2Ag_2N_4O)H_2O$, and is used in the quantitative estimation of hypoxanthine. Hypoxanthine picrate is soluble with difficulty, but if a boiling-hot solution of it is treated with a neutral or only faintly acid solution of silver nitrate the hypoxanthine is almost quantitatively precipitated as the compound $C_5H_3AgN_4O.C_6H_2(NO_2)_3OH$. Hypoxanthine does not yield an insoluble compound with metaphosphoric acid. When treated, like xanthine, with nitric acid, it yields, an almost colorless residue which, on warming with alkali, does not turn red. Hypoxanthine does not give WEIDEL'S reaction. After the action of hydrochloric acid and zinc upon a hypoxanthine solution, followed by the addition of an excess of alkali a ruby-red color develops, followed by a brownish-red color (KOSSEL). According to E. FISCHER [1] a red coloration occurs even in the acid solution.

Adenine, $C_5H_5N_5$, $=HC(N{=}C.NH_2)C(=)NH\diagdown\!\!\diagup CH(=)N{-}C{-}N$ (6-aminopurine), was first found by KOSSEL [2] in the pancreas. It occurs in all nucleated cells, but in greatest quantities in the sperm of the carp and in the thymus. Adenine has also been found in lucæmic urine (STADTHAGEN [3]). It may be obtained in large quantities from tea-leaves.

Adenine crystallizes with 3 molecules of water of crystallization in long needles which gradually become opaque in the air, but much more rapidly when warmed. If the crystals are warmed slowly with a quantity of water insufficient for solution, they suddenly become cloudy at 53° C., a characteristic reaction for adenine. It dissolves in 1086 parts cold water, but is easily soluble in warm. It is insoluble in ether, but somewhat soluble in hot alcohol and easily so in acids and alkalies. It is more easily soluble in ammonia solution than guanine, but less soluble than hypoxanthine. The silver compound of adenine is soluble with difficulty in warm nitric acid, and deposits on cooling as a crystalline mixture of adenine silver nitrates. With picric acid adenine forms a compound, $C_5H_5N_5.C_6H_2(NO_2)_3OH$, which is very insoluble but separates more readily than the hypoxanthine picrate, and can be used in the quantitative estimation of adenine. We also have an adenine mercury-picrate. Metaphosphoric acid with adenine gives a precipitate which dissolves in an excess of the acid if the solution is not too dilute. Adenine hydrochloride gives with gold chloride a double compound

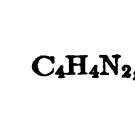
[1] Kossel, Zeitschr. f. physiol. Chem., **12**, 252; E. Fischer, l. c.
[2] See Zeitschr. f. physiol. Chem., **10** and **12**.
[3] Virchow's Arch., **109**.

which consists in part of leaf-shaped aggregations and in part of cubical or prismatic crystals, often with rounded corners. This compound is used in the microscopic detection of adenine. With the nitric-acid test and with WEIDEL'S reaction adenine acts in the same way as hypoxanthine. The same is true for its behavior with hydrochloric acid and zinc with subsequent addition of alkali.

The procedure for the preparation and detection of the four above-described purine bases is as follows: Boiling with 0.5–1 vol. per cent sulphuric acid, saturating with baryta-water, removing the excess of barium with CO_2, precipitating all the bases as silver compounds from the strongly ammoniacal filtrate and dissolving them in boiling nitric acid when the xanthine compound remains in solution on cooling while the combination with the other three bases precipitate. The silver xanthine may be precipitated from the filtrate by the addition of ammonia and the xanthine set free by means of sulphureted hydrogen. The three above-mentioned silver-nitrate compounds are decomposed by sulphureted hydrogen and the guanine separated from the adenine and hypoxanthine by treatment while hot with ammonia, in which the guanine is soluble with difficulty. When the above filtrate containing the adenine and hypoxanthine, which has been, if necessary, freed from ammonia by evaporation, is allowed to cool, the adenine separates, while the hypoxanthine remains in solution. According to BALKE [1]—we can advantageously precipitate the purine bases with copper sulphate and hydroxylamine. Details for the above methods may be found in complete hand-books. The same procedures are followed in the quantitative estimation of the purine bases in animal organs.[2]

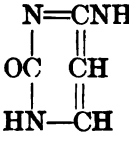
3. Pyrimidine Bases.

These bodies are closely related to the purines, and pyrimidine,



$C_4H_4N_2$, may be considered as the mother substance thereof.

The pyrimidine bases contained in the nucleic acids are cytosine, uracil and thymine.

Cytosine, $C_4H_5N_3O$,  (6 amino-2 oxypyrimidine), was first

prepared by KOSSEL and NEUMANN from thymus nucleic acid, and then by KOSSEL and STEUDEL and others from various nucleic acids. WHEELER

[1] See footnote 1, p. 190.

[2] See Burian and Hall, Zeitschr. f. physiol. Chem., **38**; Kossel *ibid.*, **5-8**, Bruhns, *ibid.*, **14**; His and Hagen, *ibid.*, **30**.

and JOHNSON[1] have also prepared it synthetically. It is transformed into uracil by the action of nitrous acid.

The free base is soluble with difficulty in water (129 parts) and crystallizes in thin leaves with a mother-of-pearl luster. It is insoluble in ether and soluble with difficulty in alcohol. The double compound with platinum chloride, the crystalline picrate, the nitrate, and the picrolonate are of importance in the detection of cytosine. This base is precipitated by phosphotungstic acid and by silver nitrate in the presence of an excess of barium hydroxide, which fact is of importance in the detection of cytosine (KUTSCHER). The double bismuth-potassium iodide gives a brick-red precipitate. Cytosine gives the murexid reaction with chlorine-water and ammonia (see Chapter XIV), and also the reaction described by WHEELER and JOHNSON under uracil. In regard to preparation see KOSSEL and STEUDEL[2] and also KUTSCHER.[3]

Uracil, $C_4H_4N_2O_2$, $=\begin{array}{ccc} HN & - & CO \\ | & & | \\ OC & & CH \\ | & & \| \\ HN & - & CH \end{array}$ (2, 6-dioxypyrimidine), was first obtained by ASCOLI and KOSSEL from yeast nucleic acid and later from various complex nucleic acids, perhaps secondarily from the cytosine as a cleavage product. The synthetical preparation was first accomplished by E. FISCHER and ROEDER.[4]

Uracil crystallizes in needles which cluster in rosettes. On careful heating it sublimes in part undecomposed, but develops red vapors and decomposes in part. It is readily soluble in hot water, but less so in cold water, and nearly insoluble in alcohol and in ether. Uracil is readily soluble in ammonia. It is precipitated by mercuric nitrate, but not by phosphotungstic acid. It is precipitated by silver nitrate only on the careful addition of ammonia or baryta-water. Uracil gives the WEIDEL reaction and the following reaction described by WHEELER and JOHNSON.[5] The uracil solution is treated with bromine-water until it is permanently cloudy and then treated with baryta-water, when a purple or violet-colored precipitate appears almost immediately. The coloration

[1] Amer. chem. Journ., **29**.

[2] Zeitschr. f. physiol. Chem., **37** and **38**.

[3] *Ibid.*, **38**. As it is not excluded, but rather probable according to Wheeler, that besides thymine also other related pyrimidine bases such as isocytosine, 6-amino pyrimidine and 6-oxpyrimidine can be formed in the hydrolytic cleavage of the nucleic acids, Wheeler has prepared salts and compounds of these bodies and described them as a matter of comparison, Journ. of biol. Chem., **3**.

[4] Ascoli, Zeitschr. f. physiol. Chem., **31**; Kossel and Steudel, *ibid.*, **37**; Levene, *ibid.*, **38**, **39**; Levene and Mandel, *ibid.*, **49**; E. Fischer and Roeder, Ber. d. d. chem. Gesellsch., **34**.

[5] Journ. of biol. Chem., **3**.

varies with the dilution. This reaction which, as remarked above, is also given by cytosine, depends upon the fact that dibromoxyhydrouracil is first formed, and from this, by the action of the barium hydroxide, first isodialuric and then dialuric acid is produced, both of which give the coloration. In regard to the preparation of uracil see KOSSEL and STEUDEL.[1]

Thymine, $C_5H_6N_2O_2$, $=\begin{array}{l} HN—CO \\ \;\;| \quad\;\; | \\ OC \quad C.CH_3 \\ \;\;| \quad\;\; | \\ HN—CH \end{array}$ (5-methyluracil). This body, which is identical with *nucleosin* obtained by SCHMIEDEBERG from salmo-nucleic acid, was first prepared by KOSSEL and NEUMANN from thymus-nucleic acid, and then by other investigators, especially LEVENE and MANDEL, from other animal nucleic acids. FISCHER and ROEDER and later GERNGROSS[2] have prepared it synthetically.

Thymine crystallizes in small leaves grouped in stellar or dendriform clusters or, rarely, in short needles (GULEWITSCH[3]). It deflagrates at 318° C. and melts at about 321° and sublimes. It is soluble with difficulty in cold water, more soluble in hot water, and insoluble in alcohol. It behaves like uracil toward ammonia or baryta-water and silver nitrate. Thymine is precipitated by phosphotungstic acid, especially when impure. Bromine-water is decolorized by thymine, producing bromthymine. For its detection we make use of the sublimation, the behavior toward silver nitrate, and its elementary analysis.

MEYERS[4] has prepared compounds of pyrimidine bases with several metals such as K, Na, Pb, Hg and he considers it incorrect to call the pyrimidine bodies bases.

In regard to the methods of preparation see KOSSEL and NEUMANN and W. JONES.[5]

The purine and pyrimidine bodies stand in close chemical and physiological relation to each other and for this reason the question has been repeatedly raised whether the pyrimidine bases might not be formed, at least in part, from the purine bases by the action of acids. Thus far no conclusive investigations have been made supporting this view, while on the contrary the investigations of STEUDEL[6] seem to contradict such a view.

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[1] Zeitschr. f. physiol. Chem., **37**.

[2] Schmiedeberg, Arch. f. exp. Path. u. Pharm., **37**; Kossel and Neumann, Ber. d. d. chem. Gesellsch., **26** and **27**; Mandel and Levene, Zeitschr. f. physiol. Chem., **46** **47**, **49**, **50**; E. Fischer and Roeder, *ibid.*, **34**; Gerngross, *ibid.*, **38**.

[3] Zeitschr. f. physiol. Chem., **27**.

[4] Journ. of biol. Chem., **7**.

[5] Kossel and Neumann, l. c., and W. Jones, Zeitschr. f. physiol. Chem., **29**, 461.

[6] Zeitschr. f. physiol. Chem., **51** and **53** (against Burian).

CHAPTER III.

THE CARBOHYDRATES.

We designate by this name bodies which are especially abundant in the plant kingdom. As the protein bodies form the chief portion of the solids in animal tissues, so the carbohydrates form the chief portion of the dry substance of the plant structure. They occur in the animal kingdom only in proportionately small quantities, either free or in combination with more complex molecules, forming compound proteins. Carbohydrates are of extraordinarily great importance as food for both man and animals.

The carbohydrates contain only *carbon*, *hydrogen*, and *oxygen*. The last two elements occur, as a rule, in the same proportion as they do in water, namely, 2:1, and this is the reason why the name carbohydrates has been given to them. This name is not quite pertinent, if strictly considered, because we not only have bodies, such as acetic acid and lactic acid, which are not carbohydrates and still have their oxygen and hydrogen in the same proportion as in water, but we also have a sugar (the methyl pentoses, $C_6H_{12}O_5$) which has these two elements in another proportion. At one time it was thought possible to characterize as carbohydrates those bodies which contained 6 atoms of carbon, or a multiple, in the molecule, but this is not considered tenable at the present time. We have true carbohydrates containing less than 6, and also those containing 7, 8, and 9 carbon atoms in the molecule.

The carbohydrates have no properties or characteristics in general which differentiate them from other bodies; on the contrary, the various carbohydrates are in many cases very different in their external properties. Under these circumstances it is very difficult to give a positive definition for the carbohydrates.

From a chemical standpoint we can say that all carbohydrates are aldehyde or ketone derivatives of polyhydric alcohols. The simplest carbohydrates, the simple sugars or monosaccharides, are either aldehyde or ketone derivatives of such alcohols, and the more complex carbohydrates seem to be derived from these by the formation of anhydrides. It is a fact that the more complex carbohydrates yield two or even more molecules of the simple sugars when made to undergo hydrolytic splitting.

Correspondingly the carbohydrates can be divided into three chief groups, namely, 1. *Simple sugars* or monosaccharides, 2. *Complex sugars* or disaccharides, trisaccharides and crystalline polysaccharides, and 3. Non-crystalline or *colloid polysaccharides*. Of these groups the monosaccharides, disaccharides and colloid polysaccharides are of special physiological importance.

Our knowledge of the carbohydrates and their structural relationships has been very much extended by the pioneering investigations of KILLIANI [1] and especially those of E. FISCHER.[2]

As the carbohydrates occur chiefly in the plant kingdom it is naturally not the place here to give a complete discussion of the numerous carbohydrates known up to the present time. According to the plan of this work it is only possible to give a short review of those carbohydrates which occur in the animal kingdom or are of special importance as food for man and animals.

1. Monosaccharides.

All varieties of sugars are characterized by the termination "*ose*," to which a root is added signifying their origin or other relations. According to the number of carbon atoms contained in the molecule the monosaccharides are divided into, *trioses*, *tetroses*, *pentoses*, *hexoses*, *heptoses*, and so on.

All monosaccharides are either aldehydes or ketones of polyhydric alcohols. The former are termed *aldoses* and the latter *ketoses*. Ordinary glucose is an aldose, while ordinary fruit sugar (fructose) is a ketose. The difference may be shown by the structural formulæ of these two varieties of sugar:

$$\text{Glucose} = CH_2(OH).CH(OH).CH(OH).CH(OH).CH(OH).CHO;$$
$$\text{Fructose} = CH_2(OH).CH(OH).CH(OH).CH(OH).CO.CH_2(OH).$$

A difference is also observed on oxidation. The aldoses can be converted into oxyacids having the same quantity of carbon, while the ketoses yield acids having less carbon. On mild oxidation the aldoses yield monobasic oxyacids, and dibasic acids on more energetic oxidation. Thus ordinary glucose yields gluconic acid in the first case and saccharic acid in the second.

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[1] Ber. d. deutsch. chem. Gesellsch., 18, 19, and 20.

[2] See E. Fischer's lecture, Synthesen in der Zuckergruppe, Ber. d. deutsch. chem. Gesellsch., 28, 2114. Excellent works on carbohydrates are Tollen's Kurzes Handbuch der Kohlehydrate, Breslau, 2 (1895), and 1, 2. Auflage, 1898, which gives a complete review of the literature, and E. O. v. Lippmann, Die Chemie der Zuckerarten, Braunschweig, 1904.

Gluconic acid = $CH_2(OH).[CH(OH)]_4.COOH$;
Saccharic acid = $COOH.[CH(OH)]_4.COOH$.

The monocarboxylic acids are easily transformed into their anhydrides (lactones), and these latter are of special interest because, as shown by FISCHER, they can be changed into the corresponding aldehyde, i.e., the corresponding aldose, by nascent hydrogen.

The monosaccharides are converted into the corresponding polyhydric alcohols by nascent hydrogen. Thus ARABINOSE, which is a pentose, $C_5H_{10}O_5$, is transformed into the pentatomic alcohol, ARABITE, $C_5H_{12}O_5$. The three hexoses, GLUCOSE, MANNOSE, and GALACTOSE, $C_6H_{12}O_6$, are transformed into the corresponding three hexites, SORBITE, MANNITE, and DULCITE, $C_6H_{14}O_6$. The ketoses, on the contrary, due to their constitution, yield a mixture of two alcohols on the same treatment. From *d*-fructose, for example, we obtain a mixture of *d*-sorbite and *l*-mannite. On careful oxidation of the polyhydric alcohols the corresponding sugar can be prepared.

Numerous isomers occur among the monosaccharides, and especially in the hexose group. In certain cases, as, for instance, in glucose and fructose, we are dealing with a different constitution (aldoses and ketoses), but in most cases we have stereoisomerism due to the presence of asymmetric carbon atoms.

As the monosaccharides from the trioses upward contain asymmetric carbon atoms they occur as optically active bodies in an *l*-, *d*,- and racemic form, *r* or *d*-*l* form, which is a mixture or a combination of the *l*- and *d*-form in equal parts. As the number of asymmetric carbon atoms increases so does the number of possible stereoisomeric forms enlarge. As the number according to VAN'T HOFF is 2^n, where n represents the number of asymmetric carbon atoms, then for the aldo-hexose, which contains 4 asymmetric carbon atoms, $2^4 = 16$ stereo-chemically different forms can exist. In fact, of these, 12 have been prepared and their geometric structure has been explained and for which FISCHER has given configuration formulæ.

As these relations are readily conceived we will, for example, give only the configuration formulæ for the most important pentoses and hexoses occurring in the animal body.

COH	COH	COH	COH
HOCH	HCOH	HOCH	HCOH
HCOH	HOCH	HCOH	HOCH
HCOH	HOCH	HOCH	HCOH
CH_2OH	CH_2OH	CH_2OH	CH_2OH
d-Arabinose	*l*-Arabinose	*d*-Xylose	*l*-Xylose

COH HĊOH HĊOH HĊOH ĊH$_2$OH *d*-Ribose	COH HOĊH HOĊH HOĊH ĊH$_2$OH *l*-Ribose	COH HĊOH HOĊH HĊOH HĊOH ĊH$_2$OH *d*-Glucose	COH HOĊH HĊOH HOĊH HOĊH ĊH$_2$OH *l*-Glucose
COH HĊOH HOĊH HOĊH HĊOH ĊH$_2$OH *d*-Galactose	COH HOĊH HĊOH HĊOH HOĊH ĊH$_2$OH *l*-Galactose	CH$_2$OH ĊO HOĊH HĊOH HĊOH ĊH$_2$OH *d*-Fructose	CH$_2$OH ĊO HĊOH HOĊH HOĊH ĊH$_2$OH *l*-Fructose

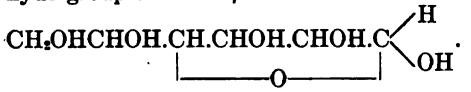
We designate the optical activity of the carbohydrates with the letter *l*- for levogyrate, *d*- for dextrogyrate, and *r*- for the racemic. These are only partly indicative. Thus dextrorotatory glucose is designated *d*-glucose, levorotatory *l*-glucose, but EMIL FISCHER has used these signs in another sense. He designates by these signs the mutual relationship of the various kinds of sugars instead of their optical activity. For example, he does not designate the levorotatory fructose *l*-fructose, but *d*-fructose, showing its close relation in stereometric structure to dextrorotatory *d*-glucose. This designation is generally accepted, and the above-mentioned signs only show the optical properties in certain cases.

Specific rotation means the rotation in degrees produced by 1 gm. substance dissolved in 1 cc. liquid placed in a tube 1 dcm. long. The reading is ordinarily made at 20° C. and with the monochromatic sodium light. The specific rotation with this light is represented by $(\alpha)_D$, and is expressed by the following formula: $(\alpha)_D = \pm\frac{a}{p.l}$, in which a represents the reading of degrees, l the length of the tube in decimeters, and p the weight of substance in 1 cc. of the liquid. Inversely the per cent P of substance can be calculated, when the specific rotation is known, by the formula $P = \frac{100a}{s.l}$, in which s represents the known specific rotation.

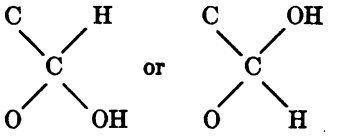
In the determination of the change in specific rotation with various concentrations we must know also the amount of substance in grams in 1 gram of the solution (p) and the specific gravity of the solution (d) at 20°. The rotation is calculated according to the formula $(\alpha)_D = \pm\frac{a}{p.l.d.}$.

A freshly prepared solution of a substance often shows a different rotation from one that has been allowed to stand for some time (multirotation). The correct values which are found on allowing the solution to stand for a sufficiently long time can be obtained immediately by boiling or on the addition of very little ammonia.

In order to explain multirotation HUDSON [1] believes that the aldoses exist in two isomeric forms having different rotation, which on being dissolved pass into each other by means of a reversible reaction. The two forms can be derived because a lactone-like binding exists between the end carbon atom in the aldehyde group and the γ-carbon atom according to the formula



. In this way the end carbon becomes asymmetric and according to whether the position of the atoms that are combined with this carbon atom are:



we obtain the two forms. TANRET [2] has obtained two isomeric forms of glucose, galactose, arabinose and lactose which pass from one form to the other. The two forms of glucose correspond according to E. H. ARMSTRONG [3] to the synthetically prepared α- and β-methyl glucosides prepared by E. FISCHER (see pages 61–62).

Like the ordinary aldehydes and ketones, the sugars may be made to take up hydrocyanic acid. Cyanhydrins are thus formed. These addition products are of special interest in that they make possible the artificial preparation of sugars rich in carbon from sugars poor in carbon.

As an example, if we start from glucose we obtain glucocyanhydrin on the addition of hydrocyanic acid:

$$CH_2.(OH).[CH(OH)]_4.COH+HCN=CH_2(OH).[CH(OH)]_4.CH(OH).CN.$$

On the saponification of glucocyanhydrin the corresponding oxyacid is formed.

$$CH_2(OH).[CH(OH)]_4.CH(OH).CN+2H_2O$$
$$=CH_2(OH).[CH(OH)]_4.CH(OH).COOH+NH_3.$$

By the action of nascent hydrogen on the lactone of this acid we obtain glucoheptose, $C_7H_{14}O_7$ and according to this principle the construction of sugars up to nine carbon atoms has been accomplished.

The monosaccharides give the corresponding oximes with hydroxylamine; thus glucose yields glucosoxime, $CH_2(OH).[CH(OH)]_4.CH:N.OH$. These compounds are of importance on account of the fact, as found by WOHL,[4] that they are the starting-point in the formation of one class

[1] Journ. Amer. Chem. Soc., **31**, 61, 955 (1909).
[2] Bull. soc. chim., **13**, 728 (1895), **15**, 195, 349 (1896).
[3] Journ. Chem. Soc., **83**, 1305 (1903).
[4] Ber. d. d. chem. Gesellsch., **26**, p. 730.

of sugars from another class, namely, the preparation of sugars poor in carbon from those rich in carbon, for example, pentoses from hexoses (see WOHL).

According to RUFF, by the action of hydrogen peroxide and the catalytic action of ferric salts upon the carbohydrate monocarboxylic acids the carbon chain can be shortened by the splitting off of the elements of formic acid, and with the formation of the next lower aldose. NEUBERG [1] has accomplished the same result by electrolysis, and by this method has split glucose step by step into formaldehyde.

By the action of alkalies, even in small amounts, as also of carbonates and lead hydroxide, a reciprocal transformation of the sugars, such as *d*-glucose, *d*-fructose, and *d*-mannose, may take place (LOBRY DE BRUYN and ALBERDA VAN EKENSTEIN [2]), and from each of these three varieties of sugar the two others are produced so that after a certain time the solution contains all three sugars.

The transformation of the different varieties of sugar into each other also occurs in the animal body. NEUBERG and MAYER [3] have shown by experiments on rabbits the direct partial transformation of various mannoses into the corresponding glucoses. Another example is, it seems, the formation of galactose (or milk sugar) from glucose in the mammary gland.

By the action of strong alkali the sugars are decomposed with the formation of lactic acid and many other products.

With ammonia the glucoses may form compounds which have been considered as osamines by LOBRY DE BRUYN, but to differentiate them from the true osamines have been called osimines by E. FISCHER.[4] The corresponding osaminic acid can be obtained from such an osimine by the action of ammonia and hydrocyanic acid, and from the hydrochloric-acid lactone of this acid the osamine is obtained by reduction with sodium amalgam. In this manner E. FISCHER and LEUCHS artificially prepared first *d*-arabinosimine from *d*-arabinose, then *d*-glucosaminic acid and finally from its lactone *d*-glucosamine, which occurs in the animal body. In a similar manner they [5] obtained *l*-glucosamine from *l*-arabinose.

KNOOP and WINDAUS [6] have obtained large amounts of methylimida-

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[1] Ruff, Ber. d. d. chem. Gesellsch., **31** and **32**; Neuberg, Biochem. Zeitschr., **7**.

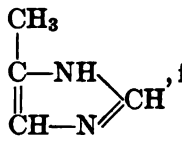
[2] Ber. d. d. chem. Gesellsch., **28**, 3078; Bull. soc. chim. de Paris (3), **15**; Chem. Centralbl., 1896, **2**, and 1897, **2**.

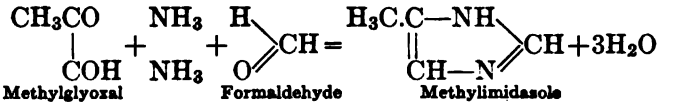
[3] Zeitschr. f. physiol. Chem., **37**.

[4] Lobry de Bruyn, Ber. d. d. chem. Gesellsch., **28**; E. Fischer, *ibid.*, **35**.

[5] *Ibid.*, **35**, p. 3787, and **36**, 24 (1903).

[6] *Ibid.*, **38**, and Hofmeister's Beiträge, **6**.

zol,  , from glucose by the action of ammonium-zinc hydroxide at ordinary temperatures. This formation can be conceived as follows: First methyl glyoxal is formed from the sugar, and then from this, or from the sugar, formaldehyde is produced, which reacts with the methyl glyoxal with the formation of methylimidazole according to the following equation:



A genetic relationship of the carbohydrates to histidine and the purine bodies is thus made probable by the imidazole formation.

As the sugars are derivatives of polyhydric alcohols, they also form esters, among which the benzoyl ester is of special interest because it is used in the detection and isolation of the sugars and also of other carbohydrates. The nucleic acids probably also belong to the acid esters of the sugars, and thus may be considered as complex phosphoric acid esters, and perhaps the chondroitin sulphuric acid and the glucothionic acid are sulphuric acid esters. The nature of these two groups of sulphuric acid esters is not yet thoroughly understood.

The sugars can also combine with other bodies and with each other, forming ether-like combinations. By the action of hydrochloric acid as catalyst, as shown by FISCHER and collaborators, the sugars split off water and unite with other bodies, producing lactone-like combinations, which have been called *glucosides* (see pages 61 and 200). These glucosides, which are generally compounds with aromatic groups, occur widely distributed in the vegetable kingdom. The more complex carbohydrates may be considered, according to FISCHER, as glucosides of the sugars. Thus maltose, for example, is the glucoside and lactose the galactoside of glucose. The glucosides can be split into their components by chemical agents, boiling with dilute mineral acids, as well as by the action of enzymes. The complex sugars hereby yield simple sugars and the other glucosides yield compounds which belong either to the aromatic or the aliphatic series besides the sugar. A long-known example of a decomposition of this kind is the splitting of amygdalin by the enzyme emulsin (see page 60).

With phenylhydrazine or substituted phenylhydrazines, the sugars first yield *hydrazones* with the elimination of water, and then on the further action of hydrazine on warming in an acetic-acid solution we obtain *osazones*.

The reaction takes place with the aldoses as follows:

(*a*) $CH_2(OH).[CH(OH)]_3.CH(OH).CHO + H_2N.NH.C_6H_5 =$
$CH_2(OH).[CH(OH)]_3.CH(OH)CH{:}N.NH.C_6H_5 + H_2O.$
Phenylglucosehydrasone

(*b*) $CH_2(OH)[CH(OH)]_3.CH(OH).CH{:}N.NH.C_6H_5 + H_2N.NH.C_6H_5 =$
$CH_2(OH).[CH(OH)]_3.C.CH{:}N.NH.C_6H_5$
$\ddot{N}.NH.C_6H_5 + H_2O + H_2.$
Phenylglucosasone

and with the keteoses:

$CH_2(OH)[CH(OH)]_3CO.CH_2(OH) + H_2N.NH.C_6H_5 =$
$CH_2(OH)[CH(OH)]_3C.CH_2OH$
$\ddot{N}.NH.C_6H_5 + H_2O,$

and $CH_2(OH)[CH(OH)]_3.C.CH_2(OH)$
$\ddot{N}.NH.C_6H_5 + H_2N.NH.C_6H_5 =$
$CH_2(OH)[CH(OH)]_3.C.CH{:}N.NH.C_6H_5 + H_2O + H_2.$
$\ddot{N}.NH.C_6H_5$

The hydrogen is not evolved, but acts on a second molecule of phenylhydrazine and splits it into aniline and ammonia:

$$H_2N.H.C_6H_5 + H_2 = H_2N.C_6H_5 + NH_3.$$

As seen from the above equations the aldoses and ketoses yield the same osazones, while the hydrazones are different.

The osazones, which are more important than the hydrazones, are generally yellow crystalline compounds which differ from each other in melting-point, solubility, and optical properties, and hence have been of great importance in the characterization of certain sugars. They have also become of extraordinarily great interest in the study of the carbohydrates for other reasons. Thus they are a very good means of precipitating sugars from solution in which they occur mixed with other bodies, and they are of the greatest importance in the artificial preparation of sugars. On cleavage, by the brief action of gentle heat and fuming hydrochloric acid (for disaccharides still better with benzaldehyde),[1] the osazones yield so-called *osones*, which on reduction yield aldoses or more often ketoses. The hydrazones can be much more readily retransformed into the corresponding sugar, especially by decomposition with benzaldehyde (HERZFELD) or with formaldehyde (RUFF and OLLENDORFF [2]), whereby the sugar is replaced by the aldehyde used.

An important property, although not applicable to all sugars, is their ability to undergo *fermentation*, especially their ability to undergo alcoholic fermentation with alcohol-yeast. We must state, however, that the power of fermentation with pure yeast has been shown only for

[1] E. Fischer and Armstrong, Ber. d. d. chem. Gesellsch., **35**.
[2] Herzfeld, *ibid.*, **28**; Ruff and Ollendorff, *ibid.*, **32**.

the hexose group, and in fact all the hexoses do not ferment, and they do not all ferment with the same readiness. *d*-Glucose and *d*-mannose ferment readily, but *d*-galactose only with difficulty. The *l*-forms of the above-mentioned sugars do not ferment, and from the racemic forms of these sugars the optical *l*-antipode can be prepared by the fermentation of the *d*-sugar. Among the ketoses the *d*-fructose ferments while the sorbose does not. Among the sugars containing nine atoms of carbon, the nonoses, the mannonose ferments while the gluconononose does not. In regard to the fermentability of the triose, dioxyacetone, see page 205. The different behavior of the various sugars with yeast stands in fixed relation to their configuration, and is not only of great importance for the behavior of the sugar in lower organisms, but also for their behavior in higher developed organisms. Thus the investigations of NEUBERG WOHLGEMUTH [1] upon arabinose and of NEUBERG and MAYER [2] on mannoses have shown that in rabbits the *l*-arabinose and the *d*-mannose are much better utilized than *d*- and *r*-arabinose or *l*- and *r*-mannose. See also pages 61–62.

In the alcoholic fermentation the sugar is decomposed according to the general equation $C_6H_{12}O_6 = 2C_2H_6O + 2CO_2$. The exact process is not clear, and seems to be rather complicated. On page 52 it has been mentioned that for the action of the fermentation enzymes the presence of a dialyzable substance present in the press-juice is necessary (HARDEN and YOUNG [3]). On the other hand the fermentation power of the press-juice can also be considerably increased by the addition of secondary sodium phosphate. The phosphoric acid in the press-juice after fermentation is only partly precipitable with magnesia mixture (HARDEN and YOUNG). The most favorable action of boiled press-juice is inhibited by lipase (BUCHNER and KLATTE). According to HARDEN and YOUNG we must consider the action of boiled press-juice and of phosphate in that first a hexose-phosphoric acid ester is produced with the simultaneous formation of carbon dioxide and alcohol, according to the following formula:

$$2C_6H_{12}O_6 + 2R_2HPO_4 = 2CO_2 + 2C_2H_6O + C_6H_{10}O_4(PO_4R_2)_2 + 2H_2O.$$

The hexose phosphate can then be split into hexose and phosphate by a special enzyme. The hexose phosphoric acid has been isolated as a lead salt by YOUNG. Glucose, fructose and mannose produce in their fermentation the same hexose phosphoric acid. According to

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[1] Zeitschr. f. physiol. Chem., **35**.

[2] *Ibid.*, **37**.

[3] Literature in Harden and Young, Bioch. Zeitschr., **32**, 173 (1911) as well as Buchner and Klatte, *ibid.*, 8, 520 (1908).

IWANOFF [1] the phosphoric acid combination is a triose phosphate which is fermented, with the formation of carbon dioxide, alcohol and phosphoric acid, by the dead and not by the living yeast. On the contrary LEBEDEW finds the same formula as YOUNG [2] for the phosphoric acid ester. IWANOFF as well as EULER and their collaborators admit that the formation of phosphoric acid esters is brought about by a special enzyme.[3] According to IWANOFF and to LEBEDEW the sugar is first fermented after it has combined with the phosphoric acid. It seems, according to all evidence, that phosphoric acid esters of carbohydrates are formed and that these are in some way of importance for the accomplishment of the fermentation. It is not probable that in the fermentation the hexose does not directly break into alcohol and CO_2. It is generally admitted that the process takes place through intermediary steps. Lactic acid is considered as one of these, although in fact, this acid is not fermented with the formation of alcohol. Recently BUCHNER and MEISSENHEIMER [4] have proposed dioxyacetone ($HOCH_2.CO.CH_2OH$) as a probable intermediary step. They found that dioxyacetone was very readily fermented by press-juice in the presence of common salt and indeed with the formation of alcohol and carbon dioxide. This has been substantiated by LEBEDEW.[5] HARDEN and YOUNG disputed the possibility that dioxyacetone is an intermediary step in the alcoholic fermentation of sugar because it is more slowly fermented than the sugars.[6]

Besides ethyl alcohol and carbon dioxide there are formed in the fermentation of sugar, although in small amounts, several higher alcohols which form the so-called fusel oil. The most important constituents of fusel oil are isoamylalcohol, *d*-amylalcohol, isobutylalcohol and normal propylalcohol in varying proportions. The formation of fusel oil was ascribed for a long time to the action of bacteria until F. EHRLICH [7] found that the higher alcohols could be produced from certain amino-acids by the living activity of yeast. From an amino-acid probably the corresponding oxyacid is formed first by the splitting off of ammonia,

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[1] Centralbl. f. Bakt. **24**, 1 (1909).

[2] Bioch. Zeitschr. **36**, 248 (1911).

[3] Euler and Kullberg, Zeitschr. f. physiol. Chem., **74**, 15 (1911); **80**, 175 (1912); Bioch. Zeitschr., **37**, 133 (1911).

[4] In reference to the intermediary products in alcoholic fermentation see Buchner and Meissenheimer, Ber. d. d. chem. Gesellsch., **43**, 1773 (1910) which also contains the literature.

[5] Compt. Rend., **153**, 136 (1911).

[6] Bioch. Zeitschr., **40**, 458 (1912).

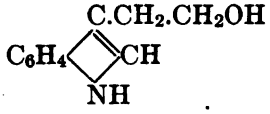
[7] Zeitschr. f. Ver. d. d. Zuckerind, **55**, 539 (1905) also Ber. d. d. chem. Gesellsch, **40**, 1027, 2538 (1907); Bioch. Zeitschr. **1**, 8 (1906); **8**, 438 (1908); **18**, 391 (1909).

and then from this by loss of CO_2 the alcohol is derived. The ammonia is assimilated by the yeast. If the amino-acid is in the racemic form then only the one component occurring naturally is transformed into alcohol while the other remains in great part unchanged. In this manner leucine is converted into isoamylalcohol according to the following equation:

$$\underset{\text{Leucine}}{HOCO.CH(NH_2).CH_2.CH}\begin{matrix}CH_3\\CH_3\end{matrix} + H_2O =$$

$$NH_3 + CO_2 + \underset{\text{Isoamylalcohol}}{HOCH_2.CH_2.CH}\begin{matrix}CH_3\\CH_3\end{matrix}$$

Other examples of the same kind is the formation of *d*-amylalcohol from *d*-isoleucine and of isobutylalcohol from α-amino-valeric acid. The formation of higher alcohols takes place with yeast poor in nitrogen and in the presence of large amounts of sugar. In an analogous manner, under the influence of yeast in the presence of sugar and inorganic nutritive salts, from tyrosine *tyrosol* (*p*-oxyphenylethyl alcohol) $HO.C_6H_4.CH_2.CH_2.OH$ is derived and from tryptophane we get *tryptophol* (β-indoxylethyl alcohol)[1].



Other fermentation processes which are brought about by yeast but without the presence of sugar have been studied by NEUBERG [2] and his collaborators. Among these we will mention the decomposition of pyroracemic acid (pyruvic acid) into carbon dioxide and acetaldehyde:

$$HO.CO.CO.CH_3 = CO_2 + HOC.CH_3.$$

The enzyme active in this fermentation is called *carboxylase*. If the pyroracemic acid exists in the form of an alkali salt then the cleavage takes place according to the formula,

$$2KO.CO.CO.CH_3 + H_2O = CO_2 + 2HOC.CH_3 + K_2CO_3$$

and alkali carbonate is formed from a neutral salt. In this case the aldehyde is condensed by the alkali to aldol, the first polymerization product of acetaldehyde.

The previously mentioned (page 41) lactic acid fermentation of various sugars is caused by the action of different varieties of bacteria. The equation represents a cleavage of one hexose molecule into two lactic acid molecules $C_6H_{12}O_6 = 2HOCO.\overset{*}{C}H(OH).CH_3$. Nothing positive

[1] Ber. d. d. chem. Gesellsch., **44**, 139 (1910); **45**, 883 (1912).

[2] Bioch. Zeitschr., **31**, 170 (1910); **32**, 323; **36**, 60, 68, 76 (1911); **47**, 405, 413 (1912).

is known as to how this cleavage occurs. According to BUCHNER and MEISSENHEIMER[1] the fermentation with the enzymes contained in the bacteria produces chiefly the racemic, inactive form of the acid. This also occurs as a rule by the action of living bacteria. In reference to the formation of lactic acid within the organism see Chapter X.

The monosaccharides are colorless and odorless bodies, neutral in reaction, with a sweet taste, readily soluble in water, generally soluble with difficulty in absolute alcohol, and insoluble in ether. Some of them crystallize well in the pure state. They are strong reducing substances. They reduce metallic silver from ammoniacal silver solutions and they also reduce other metallic oxides such as copper, bismuth and mercury oxides, on heating in alkaline solution. This behavior is of great importance in the detection and quantitative estimation of the sugars.

The simple varieties of sugar occur in part in nature as such, already formed, which is the case with both of the very important sugars, glucose and fructose. They also occur in great abundance in nature as more complex carbohydrates (di- and polysaccharides); also as ester-like combinations with different substances, as so-called glucosides.

Among the groups of monosaccharides known at the present time, those containing less than five and more than six carbon atoms in the molecule have no great importance in biochemistry, although they are of high scientific interest. Of the two groups the hexoses are the more abundant and are of special interest. The pentoses are becoming of greater importance, not only for the chemistry of plants, but also for the chemical processes in the animal body.

Pentoses ($C_5H_{10}O_5$).

As a rule the pentoses do not occur as such in nature. They are obtained from animal tissues, organs and fluids as cleavage products of the nucleic acids, or nucleoproteins. The pentoses are chiefly obtained from the plant kingdom, where nucleic acids also occur, by the hydrolytic cleavage with dilute mineral acids, of more complex carbohydrates, the so-called pentosans. The *pentosans* exist very widely distributed in the plant kingdom, and are of especially great importance in the building up of certain plant constituents. Methyl pentosans and methyl pentoses also occur in the plants, and of these, the methyl pentose, rhamnose, which occurs in several glucosides, must be specially mentioned.

The pentoses were first found in the animal kingdom by SALKOWSKI and JASTROWITZ in the urine of a person addicted to the morphine habit,

[1] Ann. d. Chem. u. Pharm., **349**, 125 (1906).

and later by SALKOWSKI and others in human urine. Small quantities of pentoses have been detected by KÜLZ and VOGEL[1] in the urine of diabetics, as also in dogs with pancreas diabetes or phlorhizin diabetes. Pentose has also been found by HAMMARSTEN among the cleavage products of a nucleoprotein obtained from the pancreas, or from the corresponding guanylic acid, and seems also, according to the observations of BLUMENTHAL, to be a constituent of nucleoproteins of various organs, such as the thymus, thyroid, brain, spleen, and liver. Their occurrence in the nucleic acids has been previously discussed. In regard to the quantity of pentoses found in the various organs, we must refer to the works of GRUND and of BENDIX and EBSTEIN and MANCINI.[2]

The pentosans (STONE, SLOWTZOFF) as well as the pentoses are of the greatest importance as foods for herbivorous animals. In regard to the value of the pentoses, the researches of SALKOWSKI, CREMER, NEUBERG, and WOHLGEMUTH[3] upon rabbits and hens show that these animals can utilize the pentoses. The question whether the pentoses are active as glycogen-formers is still an open one (see Chapter VII). The pentoses seem to be absorbed by human beings and in part utilized, but they pass in part into the urine even when small quantities are taken.[4]

The natural pentoses are reducing aldoses, and as a rule do not belong to the sugars fermentable by yeast. Still, the observations of SALKOWSKI, BENDIX, SCHÖNE and TOLLENS seem to indicate that the pentoses are fermentable.[5] They are readily decomposed by putrefaction bacteria. With phenylhydrazine and acetic acid they give crystalline osazones which are soluble in hot water, and whose melting-points and optical behavior are important for the detection of the pentoses. On heating with hydrochloric acid they yield furfurol, but no levulinic acid. In this reaction furfuran is formed from the pentose molecule, and then

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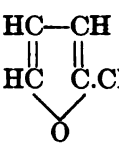
[1] Salkowski and Jastrowitz, Centralbl. f. d. med. Wissensch., 1892, 337 and 593; Salkowski, Berl. klin. Wochenschr., 1895; Bial, Zeitschr. f. klin. Med., **39**; Bial and Blumenthal, Deutsch. med. Wochenschr., 1901, No. 2; Külz and Vogel, Zeitschr. f. Biologie, **32**.

[2] Hammarsten Zeitschr. f. physiol. Chem., **19**; also Salkowski, Berl. klin. Wochenschr., 1895; Blumenthal, Zeitschr. f. klin. Med., **34**; Grund, Zeitschr. f. physiol. Chem. **35**; Bendix and Ebstein, Zeitschr. f. allgemein. Physiol., **2**; Mancini, Chem. Centralbl., 1906.

[3] Stone, Amer. Chem. Journ., **14**; Slowtzoff, Zeitschr. f. physiol. Chem., **34**; Salkowski, *ibid.*, **32**; Cremer, Zeitschr. f. Biologie, **29** and **42**; Neuberg and Wohlgemuth, Zeitschr. f. physiol. Chem., **35**.

[4] See Ebstein, Virchow's Arch., **129**; Tollens, Ber. d. deutsch. chem. Gesellsch., **29**, 1208; Cremer, l. c.; Lindemann and May, Deutsch. Arch. f. klin. Med., **56**; Salkowski, Zeitschr. f. physiol. Chem., **30**.

[5] Salkowski, Zeitschr. f. physiol. Chem., **30**; Bendix, see Chem. Centralbl., 1900, **1**; Schöne and Tollens, *ibid.*, 1901, **1**.

from this its aldehyde, the furfurol  The furfurol passing over, on distilling with hydrochloric acid, can be detected by the aid of aniline-acetate or xylidine acetate paper, which is colored a beautiful red by furfurol. In the quantitative estimation we can use the method suggested by TOLLENS, which consists in converting the furfurol in the distillate into phloroglucide by means of phloroglucin and weighing (see TOLLENS and KRÖBER, GRUND, BENDIX and EBSTEIN), or according to JOLLES [1] by bisulphite and retitrating with iodine solution. In using these methods it must be borne in mind that glucuronic-acid compounds also yield furfurol under the same conditions. The two following pentose reactions, as suggested by TOLLENS, are especially applicable.

The **orcin-hydrochloric acid test.** Mix with the solution or the substance introduced into water an equal volume of concentrated hydrochloric acid, add some orcin in substance, and heat. In the presence of pentoses the solution becomes reddish-blue, then bluish-green, and on spectroscopic examination an absorption-band is observed between *C* and *D*. If it is cooled and shaken with amyl alcohol, a bluish-green solution which shows the same band is obtained.

The **phloroglucin-hydrochloric acid test.** This test is performed in the same manner as the above, using phloroglucin instead of orcin. The solution becomes cherry-red on heating and then becomes cloudy and hence a shaking out with amyl alcohol is very necessary. The red amyl-alcohol solution shows an absorption-band between *D* and *E*. The orcin test is better for several reasons than the phloroglucin test (SALKOWSKI and NEUBERG [2]). In regard to the use of these tests in urine examination see Chapter XIV.

Many modifications of these tests have been suggested. BRAT [3] performs the orcin reaction by the addition of NaCl and heating to only 90–95°. BIAL [4] uses a hydrochloric acid containing ferric chloride for the orcin test and claims to get a greater delicacy. On too strong and too long heating (1½–2 minutes), when using this modification, a confusion with sugars of the six carbon series may occur (BIAL, VAN LEERSUM).[5] According to R. ADLER and O. ADLER the phloroglucin and orcin tests can be made with glacial acetic acid and a few drops hydrochloric acid instead of with the hydrochloric acid alone. These investigators also use a mixture of equal volumes of aniline and glacial acetic acid as a reagent for pentoses. On the addition of a little pentose to the boiling mixture a beautiful red color of furfurol-aniline acetate is obtained. A. NEUMANN [6] performs the orcin test with glacial acetic acid and adds concentrated sulphuric acid drop by drop. On following the exact instructions not only do the pentoses give this reaction, but also glucuronic acid, glucose, and fructose give characteristic

[1] Bendix and Ebstein, l. c., which contains the literature; Jolles, Ber. d. d. chem. Gesellsch., **39** and Zeitschr. f. anal. Chem., **46**.

[2] Salkowski, Zeitschr. f. physiol. Chem., **27**; Neuberg, *ibid.*, **31**.

[3] Zeitschr. f. klin. Med., **47**.

[4] Deutsch. med. Wochenschr., 1902 and 1903, and Zeitschr. f. klin. Med., **50**.

[5] Bial, Zeitschr. f. klin. Med., **50**; van Leersum, Hofmeister's Beiträge, **5**.

[6] R. and O. Adler, Pflüger's Arch., **106**; A. Neumann, Berl. klin. Wochenschr., 1904.

colored solutions with special absorption-bands which can be made use of in identifying the various sugars. Fr. Sachs has tested Bial's test and has given special precautions to prevent confusion with glucuronic acid. Jolles [1] precipitates (from urine) the pentoses as osazones, distills the precipitate with hydrochloric acid, and tests the distillate with Bial's reagent.

In performing the above two tests for pentose it must be borne in mind that glucuronic acid gives the same reactions and also that the colors alone are not sufficient. The spectroscopic examination must therefore never be omitted. Both tests are to be considered as tests of detection rather than definite pentose reactions, and therefore for a positive detection of pentoses we must prepare also the osazones or other compounds.

Arabinoses. The pentose isolated by Neuberg from human urine is *r*-arabinose. It can be isolated from the urine as the diphenylhydrazone, from which the arabinose can be separated by splitting with formaldehyde. The inactive *r*-arabinose seems to be the pentose regularly occurring in pentosuria and thus far, in only one case, has *l*-arabinose been found. *l*-Arabinose is said to pass into the urine after partaking of certain fruits, such as plums, in large amounts (C. Barszczewski [2]).

The *r*-arabinose is crystalline, has a sweetish taste, and melts at 163–164° C. Its diphenylhydrazone, which, according to Neuberg and Wohlgemuth,[3] can be used in its quantitative estimation, melts at 206° C., is insoluble in cold water and alcohol, but readily soluble in pyridine. The osazone melts at 166–168° C.

The dextrorotatory *l*-arabinose is obtained by boiling gum arabic or cherry gum with dilute sulphuric acid. The *d*-arabinose has been prepared synthetically. The phenylosazone of *l*-arabinose melts at 160°. The *l*-arabinose which crystallizes in plates or prisms melts at about 164°. The specific rotation is $(\alpha)_D = +104.5°$.

Xyloses. The *l*-xylose occurs extensively in the plant kingdom and is prepared from wood-gum by the action of dilute acid. Xylose is crystalline, melts at 150–153° C., dissolves very readily in water but with difficulty in alcohol, is faintly dextrorotatory, $(\alpha)_D = +18.1°$, and gives a phenylosazone which melts at 155–158° C., and according to Tollens and Müther a diphenylhydrazone which melts at 107–108°. According to Bertrand xylose can be transformed into xylonic acid, $CH_2(OH)[CH(OH)]_3COOH$, by bromine-water and the brom-cadmium compound or the brucine salt (Neuberg) of this acid is well suited for the detection and isolation of *l*-xylose. On oxidation with nitric acid the optically inactive trioxyglutaric acid, with a melting-point of 152° C. is obtained.

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[1] Fr. Sachs, Biochem. Zeitschr., **1** and **2**; Jolles, *ibid.*, **2**, Centralbl. f. inn. Med., 1907, and Zeitschr. f. anal. Chem., **46**.

[2] Neuberg, Ber. d. d. chem. Gesellsch., **33**; Barszczewski, Maly's Jahrsb., **27**, 733.

[3] Zeitschr. f. physiol. Chem., **35**.

According to NEUBERG and to REWALD[1] the pentose obtained from a pancreas nucleoprotein and the pentose isolated by NEUBERG and BRAHN from inosinic acid is identical with *l*-xylose.

Ribose. This pentose has been prepared synthetically by E. FISCHER. The phenylhydrazone melts at 154–155° C, the *p*-bromphenylhydrazone at 164–165° C. The osazone is identical with arabinosazone. On oxidation it yields an optically inactive trioxyglutaric acid, which melts at 170–171° C. *d*-ribose is, according to LEVENE and JACOBS, the pentose of inosinic acid, guanylic acid and yeast nucleic acid. According to these workers the pentose exists in these nucleic acids in a glucoside-like combination with the purine bases, as so-called *nucleosides*. It must be remarked that NEUBERG adheres to his claim that *l*-xylose exists at least in the pancreas.[2]

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colored solutions with special absorption-bands which can be made use of in identifying the various sugars. FR. SACHS has tested BIAL'S test and has given special precautions to prevent confusion with glucuronic acid. JOLLES [1] precipitates (from urine) the pentoses as osazones, distills the precipitate with hydrochloric acid, and tests the distillate with BIAL'S reagent.

In performing the above two tests for pentose it must be borne in mind that glucuronic acid gives the same reactions and also that the colors alone are not sufficient. The spectroscopic examination must therefore never be omitted. Both tests are to be considered as tests of detection rather than definite pentose reactions, and therefore for a positive detection of pentoses we must prepare also the osazones or other compounds.

Arabinoses. The pentose isolated by NEUBERG from human urine is *r*-arabinose. It can be isolated from the urine as the diphenylhydrazone, from which the arabinose can be separated by splitting with formaldehyde. The inactive *r*-arabinose seems to be the pentose regularly occurring in pentosuria and thus far, in only one case, has *l*-arabinose been found. *l*-Arabinose is said to pass into the urine after partaking of certain fruits, such as plums, in large amounts (C. BARSZCZEWSKI [2]).

The *r*-arabinose is crystalline, has a sweetish taste, and melts at 163–164° C. Its diphenylhydrazone, which, according to NEUBERG and WOHLGEMUTH,[3] can be used in its quantitative estimation, melts at 206° C., is insoluble in cold water and alcohol, but readily soluble in pyridine. The osazone melts at 166–168° C.

The dextrorotatory *l*-arabinose is obtained by boiling gum arabic or cherry gum with dilute sulphuric acid. The *d*-arabinose has been prepared synthetically. The phenylosazone of *l*-arabinose melts at 160°. The *l*-arabinose which crystallizes in plates or prisms melts at about 164°. The specific rotation is $(\alpha)_D = +104.5°$.

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This reaction depends, according to VILLE and DERRIEN, as well as to v. EKENSTEIN and BLANKSMA [1] upon the formation of oxymethylfurfurol which reacts with the α-naphthol. As oxymethylfurfurol is formed from all hexoses, hence MOLISCH'S reaction is a general reaction for hexoses.

DIAZOBENZENESULPHONIC ACID gives with a glucose solution made alkaline with a fixed alkali a red color, which after 10–15 minutes gradually changes to violet. ORTHONITROPHENYLPROPIOLIC ACID yields indigo when boiled with a small quantity of glucose and sodium carbonate, and this is converted into indigo-white by an excess of sugar. An alkaline solution of glucose is colored deep red on being warmed with a dilute solution of PICRIC ACID. The behavior of glucose toward certain pentose reactions has been given on page 209.

A more complete description as to the performance of these several tests will be given in detail in a subsequent chapter (on the urine).

Glucose is prepared, pure, by inverting cane-sugar by the following simple method of SOXHLET and TOLLENS, which is a modification of SCHWARZ'S [2] method:

Treat 12 liters 90 per cent alcohol with 480 cc. fuming hydrochloric acid and warm to 45–50° C.; gradually add 4 kilos of powdered cane-sugar, and allow to cool after two hours, when all the sugar will have dissolved and been inverted. To incite crystallization, some crystals of anhydrous glucose are added, and after several days the crystals are sucked dry by the air-pump, washed with dilute alcohol to remove hydrochloric acid, and crystallized from alcohol or methyl alcohol. According to TOLLENS it is best to dissolve the sugar in one-half its weight of water on the water-bath and then add double this volume of 90–95 per cent alcohol.

In detecting glucose in animal fluids or extracts of tissues we may make use of the above-mentioned reduction tests, the optical determination, fermentation, and phenylhydrazine tests. For the quantitative estimation the reader is referred to the chapter on the urine. Those liquids containing proteins must first have these removed by coagulation with heat and addition of acetic acid, or by precipitation with alcohol or metallic salts, before testing for glucose. In regard to the difficulties of operating with blood and serous fluids we refer the student to larger works.

Mannoses. *d-Mannose*, also called *seminose*, is obtained with *d*-fructose on the careful oxidation of *d*-mannite. It is also obtained on the hydrolysis of natural carbohydrates, such as salep slime and reserve cellulose (especially from the shavings of the ivory-nut). It is dextrorotatory, readily ferments with beer-yeast, gives a hydrazone not readily soluble in water, and an osazone which is identical with that from *d*-glucose.

***d*-Galactose** (not to be mistaken for lactose or milk-sugar) is obtained on the hydrolytic cleavage of milk-sugar, and by the hydrolysis of many other

[1] Bull. soc. chim. (4), 5, 895 (1909); Ber. d. d. chem. Gesellsch., 43, 2358 (1910).

[2] Tollens, Handbuch der Kohlehydrate, 2. Aufl. I, 39.

carbohydrates, especially varieties of gums and mucilaginous bodies. It is also obtained on heating cerebrin, a nitrogenized glucoside prepared from the brain, with dilute mineral acids.

It crystallizes in needles or leaves which melt at 168° C. It is somewhat less soluble in water than glucose. It is dextrogyrate, and according to NEUBERG [1] has a rotation $(\alpha)_D = +81°$. With ordinary yeast galactose is slowly, but nevertheless completely, fermented. It is fermented by a great variety of yeasts (E. FISCHER and THIERFELDER), but not by Saccharomyces apiculatus,[2] which is of importance in physiological-chemical investigations. Galactose reduces FEHLING'S solution to a less extent than glucose, and 10 cc. of this solution are reduced, according to SOXHLET, by 0.0511 gram galactose in 1 per cent solution. Its phenylosazone melts according to NEUBERG at 196–197° C., and is soluble with difficulty in hot water, but with relative ease in hot alcohol. Its solution in glacial acetic acid is optically inactive. In the test with hydrochloric acid and phloroglucin galactose gives a color similar to that of the pentoses, but the solution does not give the absorption spectrum. On oxidation it first yields galactonic acid and then mucic acid, and these serve in the detection of galactose.

***d*-Fructose** (levulose) also fruit-sugar, occurs, as above stated, mixed with glucose, extensively distributed in the vegetable kingdom and also in honey. It is formed in the hydrolytic cleavage of cane-sugar and several other carbohydrates, but it is very readily obtained by the hydrolytic splitting of inulin. In extraordinary cases of diabetes mellitus we find fructose in the urine. NEUBERG and STRAUSS [3] have detected fructose with positiveness in human blood-serum, and exudates in certain cases.

Fructose crystallizes with comparative difficulty in coarse crusts or warts or in fine needles. C. MÖRNER [4] has obtained crystals 2–3 mm. long which belonged to the rhombic system, and neither melted nor lost in weight on heating to 100° C. The melting-point is 110° C. Fructose is readily soluble in water, but almost insoluble in cold absolute alcohol, though rather readily in boiling alcohol. Its aqueous solution is levogyrate. C. MÖRNER found the rotation for a 10 and 20 per cent solution was $(\alpha)_D = -93°$ and $-94.1°$ respectively. Fructose ferments with yeast, and gives the same reduction tests as glucose, and also the same osazone. It gives a compound with lime which is less soluble than the corresponding glucose compound. Fructose is not precipitated by sugar of lead or basic lead acetate.

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[1] See C. Oppenheimer, Handb. d. Biochem. **1**, p. 197.

[2] See F. Voit, Zeitschr. f. Biol., **28** and **29**.

[3] Zeitschr. f. physiol. Chem., **36**, which also contains the older literature.

[4] Svensk. Farmac. Tidskr, No. 6, 1907. See also Maly's Jahresb., **37**, p. 95.

Fructose does not reduce copper to the same extent as glucose. Under similar conditions the reduction relationship is 100 : 92.08.

In detecting fructose and those varieties of sugar which yield fructose on cleavage we make use of the following reaction, suggested by SELIWANOFF which consists in heating with hydrochloric acid and resorcinol. This depends upon the formation of oxymethylfurfurol and is therefore obtained by all hexoses. As the ketoses give about 20 per cent oxymethylfurfurol and the aldoses only 1 per cent the reaction is more readily obtained with the ketohexoses than with the aldohexoses (v. EKENSTEIN and BLANKSMA, page 216). To a few cubic centimeters of fuming hydrochloric acid add an equal volume of water and a small quantity of the sugar solution or of the solid substance and a few crystals of resorcinol, and apply heat. The liquid becomes a beautiful red, and gradually a substance precipitates which is red in color and soluble in alcohol. According to OFNER [1] the mixture must not contain more than 12 per cent HCl, and the boiling must not be continued longer than twenty seconds, if it is boiled for a longer time and with more hydrochloric acid this reaction is also given with the aldoses. R. and O. ADLER [2] perform the test with glacial acetic acid and a drop of hydrochloric acid and some resorcinol, in which case a reaction with aldoses is not obtained. SELIWANOFF'S reaction, according to ROSIN,[3] may be made more delicate by a combination with the spectroscopic examination. In regard to its use in urine examinations see Chapter XIV.

The **naphtho-resorcinol** reaction as suggested by B. TOLLENS and RORIVE [4] can be carried out as follows: A few particles of the sugar and about the same quantity of naphthoresorcinol are treated with about 10 cc. of a mixture of equal volumes of water and concentrated hydrochloric acid of sp. gr. 1.19. This is slowly heated to boiling over a low flame, and is continued for 1–3 minutes. The fluid becomes more purple or violet than with SELIWANOFF'S resorcin test. The spectroscopic examination shows a faint band in the green.

According to NEUBERG,[5] methylphenlhydrazine is an excellent substance to use for the separation and detection of fructose, as it gives a characteristic fructose methylphenylosazone. This osazone when recrystallized from alcohol melts at 153°. It shows a dextrorotation of 1° 40′ when 0.2 gram of the osazone is dissolved in 4 cc. pyridine and 6 cc. absolute alcohol.

OFNER has made objections to the use of methylphenylhydrazine in the detection of fructose. He has obtained the osazone from glucose and methylphenylhydrazine, although the osazone is formed much more quickly with fructose than with glucose. Only when the separation of the osazone crystals with methylphenylhydrazine after the addition of acetic acid takes place within five hours at ordinary temperatures is the presence of fructose positively proven (OFNER [6]).

The use of secondary asymmetric hydrazines as a general reagent for ketoses and as a means of separation from aldoses is objected to by OFNER.

***d*-Sorbinose** (sorbin) is a ketose obtained from the juice of the berry of the mountain ash under certain conditions. It is crystalline and levogyrate, and is converted into *d*-sorbite by reduction.

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[1] Monatshefte f. Chem., **25**.

[2] See footnote 6, p. 209.

[3] Ber. d. d. chem. Gesellsch., **38**.

[4] *Ibid.*, **41**, p. 1783 and Tollens, *ibid.*, **41**, p. 1788. See also Mandel and Neuberg, Biochem. Zeitschr., **18**.

[5] *Ibid.*, **35**; also Neuberg and Strauss, *ibid.*, **36**.

[6] *Ibid.*, **37**, and Zeitschr. f. physiol. Chem., **45**.

Appendix to the Monosaccharides.

a. Amino-sugars.

The most important amino-sugar is the already mentioned glucosamine.

***d*-Glucosamine** (chitosamine), $C_6H_{13}NO_5$, $= \begin{matrix} CH_2OH \\ (CH.OH)_3 \\ CH.NH_2 \\ COH \end{matrix}$, whose synthetical preparation has been given on page 201 was first prepared by LEDDERHOSE [1] from chitin by the action of concentrated hydrochloric acid. Recently it has been obtained as a cleavage product of several mucin substances and proteins (see pages 84 and 168). Glucosamine is, as E. FISCHER and LEUCHS [2] have shown, a derivative of glucose or of *d*-mannose (probably glucose), and is an α-amino-sugar.

The free base, which can crystallize in needles, is readily soluble in water giving an alkaline reaction, and quickly decomposes. The characteristic hydrochloride forms colorless crystals which are stable in the air and readily soluble in water, soluble with difficulty in alcohol, and insoluble in ether. The solution is dextrorotatory, $(\alpha)_D = +70.15°$ to $74.64°$, at various concentrations.[3] Glucosamine has a reducing action similar to that of glucose, and gives the same osazone, but is not fermentable. With benzoyl-chloride and caustic soda it gives a crystalline ester. In alkaline solution it gives with phenylisocyanate a compound which can be converted into its anhydride by acetic acid, and is used in the separation and detection of glucosamine (STEUDEL).[4] On oxidation with nitric acid it yields norisosaccharic acid, whose lead salt can be separated, and whose salts with cinchonine or quinine are soluble with difficulty in water and can also be used very successfully in the detection of glucosamine (NEUBERG and WOLFF [5]). On oxidation with bromine, chitaminic acid (*d*-glucosaminic acid) is produced, and this is converted into chitaric acid, $C_6H_{10}O_6$, by nitrous acid. On treatment with nitrous acid glucosamine yields a non-fermentable sugar called chitose.

EHRLICH [6] has suggested a test which does not respond with the free glucosamine, but with the mucins and other protein bodies containing an acetylated glucosamine. It consists in warming the substance, which has been previously

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[1] Zeitschr. f. physiol. Chem., **2** and **4**.

[2] Ber. d. d. chem. Gesellsch., **36**.

[3] See Hoppe-Seyler-Thierfelder's Handbuch, 8, Aufl.; Sundvik, Zeitschr. f. physiol Chem., **34**.

[4] Zeitschr. f. physiol. Chem., **34**.

[5] Ber. d. d. chem. Gesellsch., **34**.

[6] Mediz. Woche, 1901, No. 15; see Langstein, Ergebnisse der Physiol., I, Abt. 1, 88.

treated with alkali, with a hydrochloric-acid solution of dimethylaminobenzaldehyde, when a beautiful red color is obtained.

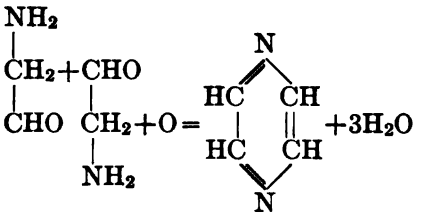
Glucosamine is best prepared from decalcified lobster-shells by treating with hot concentrated hydrochloric acid.[1] In regard to its preparation from protein substances we must refer to the works cited on page 84, footnote 5.

Albamine (diglucosamine), $(C_6H_{11}O_4N)_2+H_2O$, is the name given by S. FRÄNKEL [2] to a body which he isolated from the products of the hydrolysis of ovalbumin with baryta, as well as in its digestion. Albamine is amorphous, dextrogyrate, and reduces after boiling with acids. As hydrolytic cleavage product it yields *d*-glucosamine.

Galactosamine is claimed to have been found by SCHULZ and DITTHORN in a glycoprotein of the spawn of the frog. This claim is not generally accepted. v. EKENSTEIN and BLANKSMA [3] obtained galactose on the hydrolysis of the slimy envelope of frog eggs.

According to OFFER,[4] pentosamine occurs in the liver of the horse. According to OFFER, the pentose derivative, which he calls dipentosamine $(C_5H_7O_3.NH_2)_2+H_2O$ and a second, perhaps a diacetyl-pentosamine $2(CH_3CO)C_{10}H_{18}N_2O_7$ (?), also occur in the liver. The first gives pentose reactions and reduces FEHLING'S solution after boiling with acid. The only amino-sugar positively detected in the animal organs is glucosamine.

The amino-sugars, as intermediary bodies between the carbohydrates and oxyamino-acids, are of great physiological interest, and this interest has become still more important since NEUBERG was first able to prepare the corresponding amino-aldehyde from glycocoll and then also from other amino-acids. From the ethyl ester of glycocoll in acid solution NEUBERG [5] obtained the amino-acetaldehyde. $NH_2.CH_2.CHO$, by treatment with sodium amalgam. This aldehyde is very unstable and has a tendency to condensation with ring formation, and NEUBERG obtained therefrom by oxidation with corrosive sublimate and caustic soda, pyrazine according to the equation:



[1] See Hoppe-Seyler-Thierfelder's Handbuch, 8. Aufl.

[2] Monatsh. f. Chem., **19**.

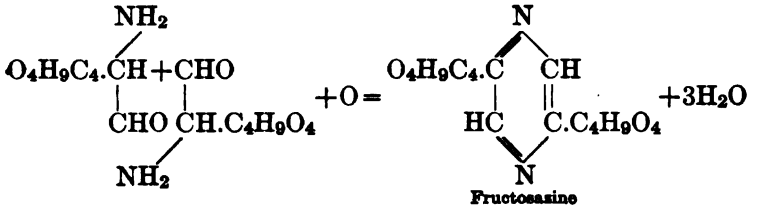
[3] Schulz and Ditthorn, Zeitschr. f. physiol. Chem., **29**; v. Ekenstein and Blanksma, Chem. Centralbl., 1907, **2**, p. 1001.

[4] Hofmeister's Beiträge, 8.

[5] Ber. d. d. chem. Gesellsch., **41**.

On account of this tendency to ring-formation the amino-acetaldehyde as well as the amino-aldehydes as a group, stand, according to NEUBERG, in close relationship to many ring systems, such as imidazole, piperazine, pyrazine, pyridine and others, and also to the alkaloids.

The amino-sugars, like the amino-aldehydes, can also unite, forming ring compounds, and this seems to be the case on the decomposition of free glucosamine in aqueous solution, which occurs with access of air (LOBRY DE BRUYN). As found by STOLTE [1] 2,5-ditetraoxybutyl pyrazine (= fructosazine) is hereby produced according to the following equation:



Fructosazine

The 2,5-ditetraoxybutyl pyrazine, which STOLTE obtained by LOBRY DE BRUYN'S [2] method from fructose in methyl alcohol solution and ammonia, and which he calls *fructosazine*, can be oxidized outside of the body into 2,5-pyrazine dicarboxylic acid.

The same acid can be formed in the animal body (rabbits), although not constantly, after introducing fructosazine. It also passes into the urine of rabbits after intravenous injection of *d*-fructose and glycocoll (SPIRO), a behavior which SPIRO claims indicates that carbohydrates in metabolism react with the cleavage products of proteins. STOLTE'S experiments to decide the question whether in the animal body the glucosamine in its decomposition passes into fructosazine did not at first yield conclusive results. His more recent investigations [3] show on the contrary that in rabbits 2-oxymethylpyrazine-5-carboxylic acid is formed as an oxidation product, and this can be oxidized outside of the body into pyrazine-2, 5-dicarboxylic acid.

b. Glucuronic Acids.

The glucuronic acids occurring in the animal body either physiologically or pathologically, are conjugated acids which will be described in detail in a subsequent chapter (XIV). We will here describe only the *d*-glucuronic acid in connection with the carbohydrates.

***d*-Glucuronic acid** (glycuronic acid), $C_6H_{10}O_7 = (\overset{CHO}{\underset{COOH}{CH.OH}})_4$, is a derivative of glucose, and has been synthetically prepared by E. FISCHER and

[1] Hofmeister's Beiträge, **11**.

[2] Cited by Stolte, Hofmeister's Beiträge, **11**.

[3] Spiro, Hofmeister's Beiträge, **10**, p. 283; Stolte, Biochem. Zeitschr., **12**.

PILOTY [1] by the reduction of the lactone of saccharic acid. On oxidation with bromine it forms saccharic acid, and on reduction it yields gulonic-acid lactone. SALKOWSKI and NEUBERG [2] have obtained *l*-xylose from glucuronic acid by splitting off CO_2 by means of putrefaction bacteria.

Glucuronic acid has not been found in the free state in the animal body. It occurs to a slight extent in normal urine as a conjugated acid (MAYER and NEUBERG). It occurs to a much greater extent in urine as conjugated acid after the ingestion of certain aromatic and also aliphatic substances, especially camphor and chloral hydrate. It was obtained first by SCHMIEDEBERG and MEYER from camphoglucuronic acid, and then by v. MERING [3] from urochloralic acid by cleavage with dilute acids. According to P. MAYER,[4] on the oxidation of glucose a partial formation of glucuronic acid and oxalic acid takes place, and therefore, according to him, an increased elimination of conjugated glucuronic acids shows in certain cases an incomplete oxidation of glucose. Conjugated glucuronic acids may also occur in the blood (P. MAYER, LÉPINE and BOULUD [5]), in the feces, and in the bile.[6] NEUBERG and NEIMANN [7] have prepared certain conjugated glucuronic acids (see Chapter XIV) synthetically, among them being euxanthic acid. The most abundant source of glucuronic acid is the artist's pigment "Jaune indien," which contains the magnesium salt of euxanthic acid (euxanthon-glucuronic acid).

Glucuronic acid is not crystalline, but is only obtainable as a syrup. It dissolves in alcohol and is readily soluble in water. If the aqueous solution is boiled for an hour the acid is partly (20 per cent) converted into the crystalline lactone, glucurone, $C_6H_8O_6$, which is soluble in water and insoluble in alcohol, and which has a melting-point of 175–178° C. The alkali salts of the acid are crystalline. If a concentrated solution of the acid is saturated with barium hydroxide the basic barium salt is obtained as a precipitate. The neutral lead salt is soluble in water, while the basic salt is insoluble. The readily crystallizable cinchonine salt can be used in isolating glucuronic acid (NEUBERG [8]). Glucuronic acid is dextrorotatory, while the conjugated acids are levorotatory; they behave like glucose with the reduction tests, and do not ferment

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[1] Ber. d. d. chem. Gesellsch., **24**.

[2] Zeitschr. f. physiol. Chem., **36**.

[3] Mayer and Neuberg, Zeitschr. f. physiol. Chem., **29**; Schmiedeberg u. Meyer, *ibid.*, **3**; v. Mering, *ibid*, **6**.

[4] Zeitschr. f. klin. Med., **47**. See Chapter XIV.

[5] Mayer, Zeitschr. f. physiol. Chem., **32**; Lépine and Boulud, Compt. rend., **132**, **134**, **138**.

[6] See Bial, Hofmeister's Beiträge, **2**, and v. Leersum, *ibid.*, **3**.

[7] Zeitschr. f. physiol. Chem., **44**.

[8] Ber. d. d. chem. Gesellsch., **33**.

with yeast. With the phenylhydrazine test it gives crystalline compounds which are not sufficiently characteristic (THIERFELDER, P. MAYER [1]). By the action of 3 mol. phenylhydrazine and the necessary amount of acetic acid upon 1 mol. glucuronic acid at 40° for a few days, NEUBERG and NEIMANN obtained the glucuronic-acid osazone, which was very similar to glucosazone and melted at 200–205°. With *p*-bromphenylhydrazine hydrochloride and sodium acetate, glucuronic acid gives *p*-bromphenylhydrazine glucuronate, which is characterized by its insolubility in absolute alcohol and by a very prominent levorotatory action. This compound is very well suited for the detection of glucuronic acid.[2] Dissolved in a mixture of alcohol and pyridine (0.2 gram substance in 4 cc. pyridine and 6 cc. alcohol) the rotation is 7° 25′, which corresponds to $(\alpha)_D^{20} = -369°$. On distillation with hydrochloric acid, glucuronic acid yields furfurol and also carbon dioxide, and on this behavior TOLLENS and LEFÈVRE [3] have based their quantitative method for the estimation of glucuronic acid.

They give the pentose reactions with phloroglucin or orcin and hydrochloric acid, and also a good reaction with naphthoresorcinol and hydrochloric acid (see page 218). The product produced herewith is soluble in ether with a blue, bluish-violet or reddish-violet color, and the solution shows an absorption band somewhat to the right and on the *D*-line. According to MANDEL and NEUBERG [4] this reaction is not characteristic of glucuronic acid, as many aldehyde and ketone acids give the same reaction; still, it is important in the differentiation of the pentoses.

Glucuronic acid is best prepared from euxanthic acid, which decomposes on heating it with water to 120° C. for several hours. The filtrate from the euxanthon is concentrated at 40° C., when the anhydride gradually crystallizes out. On boiling the mother-liquor for some time and evaporating further, the crystals of the lactone are obtained. In regard to the quantitative estimation of glucuronic acid we must refer the reader to the works of TOLLENS and his collaborators and of NEUBERG and NEIMANN.[5]

2. Disaccharides.

Some of the varieties of sugar belonging to this group occur ready formed in nature. Thus we have saccharose and lactose. Some, on the contrary, such as maltose and isomaltose, are produced by the partial

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[1] Thierfelder, Zeitschr. f. physiol. Chem., **11**, **13**, **15**; P. Mayer, *ibid.*, **29**.

[2] See Neuberg, Ber d. d. chem. Gesellsch., **32**; and Mayer and Neuberg, Zeitschr. f. physiol. Chem., **29**.

[3] Ber. d. d. chem. Gesellsch., **40**.

[4] Bioch. Zeitschr. **13**.

[5] Tollens, Zeitschr. f. physiol. Chem., **44**, which cites also the older work; Neuberg and Neimann, *ibid.*, **44**; Neuberg, *ibid.*, **45**.

hydrolytic cleavage of complex carbohydrates. Isomaltose is also obtained from glucose by reversion (see page 225).

The disaccharides or hexobioses are to be considered as glucosides, each of which is derived from two monosaccharides with the exit of 1 molecule of water. Corresponding to this, their general formula is $C_{12}H_{22}O_{11}$. On hydrolytic cleavage and the addition of water they yield 2 molecules of hexoses, either 2 molecules of the same hexose or one each of two different hexoses. Thus

$$\text{Saccharose} + H_2O = \text{glucose} + \text{fructose};$$
$$\text{Maltose} + H_2O = \text{glucose} + \text{glucose};$$
$$\text{Lactose} + H_2O = \text{glucose} + \text{galactose}.$$

The configuration of the disaccharides has not been positively determined.

The fructose turns the polarized ray more to the left than the glucose does to the right; hence the mixture of hexoses obtained on the cleavage of saccharose has an opposite rotation to the saccharose itself. On this account the mixture is called INVERT-SUGAR, and the hydrolytic splitting is designated as *inversion*. This term, "inversion," is not only used for the splitting of saccharose, but is also used for the hydrolytic cleavage of compound sugars into monosaccharides. The reverse reaction, whereby monosaccharides are condensed into complex carbohydrates, is called *reversion*.

We subdivide the disaccharides into two groups, first, the group to which saccharose belongs, where the members do not have the property of reducing certain metallic oxides; and the second group, to which the two maltoses and lactose belong, the members acting like monosaccharides in regard to the ordinary reduction tests. The members of the latter group have the character of aldehyde alcohols, and in milk-sugar the aldehyde characteristics are connected with the glucose fraction.

Saccharose, or CANE-SUGAR, occurs extensively distributed in the plant kingdom. It occurs to the greatest extent in the stalk of the sugar-millet and sugar-cane, the roots of the sugar-beet, the trunks of certain varieties of palms and maples, in carrots, etc. Cane-sugar is of extraordinary great importance as a food and condiment.

Saccharose forms large, colorless monoclinic crystals. On heating it melts in the neighborhood of 160° C., and on heating more strongly it turns brown, forming so-called caramel. It dissolves very readily in water, and according to HERZFELD,[1] 100 parts of saturated saccharose solution contain 67 parts of sugar at 20° C. It dissolves with difficulty in strong alcohol. Cane-sugar is strongly dextrorotatory. The specific rotation is only slightly modified by concentration, but is markedly

[1] See Tollens' Handbuch der Kohlehydrate, 2. Aufl. 1, 154.

changed by the presence of other inactive substances. The specific rotation is $(\alpha)_D = +66.5°$.

Saccharose acts indifferently toward MOORE'S test and to the ordinary reduction tests. On continuous boiling it may reduce an alkaline copper solution, perhaps on account of a partial inversion. It does not ferment directly, but only after inversion, which can be brought about by an enzyme (invertin) contained in the yeast. An inversion of cane-sugar also takes place in the intestinal canal. Cane-sugar does not combine with hydrazines. Concentrated sulphuric acid blackens cane-sugar very quickly even at the ordinary temperature, and anhydrous oxalic acid does the same on warming on the water-bath. Various products are obtained on the oxidation of cane-sugar, dependent upon the variety of oxidizing agent and also upon the intensity of the action. Saccharic acid and oxalic acid are the most important products.

The reader is referred to complete text-books on chemistry for the preparation and quantitative estimation of cane-sugar.

Maltose (MALT-SUGAR) is formed in the hydrolytic cleavage of starch by malt diastase, saliva, or pancreatic juice. It is obtained from glycogen under the same conditions (see Chapter VII). Maltose is also produced transitorily in the action of sulphuric acid on starch. Maltose forms the fermentable sugar of the potato or grain mash, and also of the beerwort.

Maltose crystallizes with one molecule water of crystallization in fine white needles. It is readily soluble in water, rather easily in alcohol, but insoluble in ether. Its solutions are dextrorotatory; and the specific rotation is variable, depending upon the concentration and temperature, but is considerably stronger than glucose,[1] and is generally given as $(\alpha)_D = +137$ to 138°. Maltose ferments readily and completely with yeast, and acts like glucose in regard to the reduction tests. It yields phenylmaltosazone on warming with phenylhydrazine for 1½ hours. This phenylmaltosazone melts at 205° C., and is more soluble in hot water than the glucosazone. Maltose differs from glucose chiefly in the following: It does not dissolve as readily in alcohol, has a stronger dextrorotatory power, and has a feebler reducing action on FEHLING'S solution; 10 cc. FEHLING'S solution are, according to SOXHLET,[2] reduced by 77.8 milligrams anhydrous maltose in approximately 1 per cent solution.

Isomaltose. This variety of sugar, as has been shown by FISCHER,[3] is produced, as are dextrin-like products, by reversion, and by the action of fuming hydrochloric acid on glucose. A re-formation of isomaltose

[1] See Hoppe-Seyler-Thierfelder's Handbuch, 8. Aufl.

[2] Cited from Tollens' Handbuch. der Kohlehydrate, 2. Aufl. 1, 154.

[3] Ber. d. deutsch. chem. Gesellsch., 23 and 28.

and other sugars from glucose can also be brought about by means of yeast maltase (HILL and EMMERLING, see page 58). It is also formed, besides ordinary maltose, in the action of diastase on starch paste, and occurs in beer and in commercial starch-sugar. It is produced, with maltose, by the action of saliva or pancreatic juice (KÜLZ and VOGEL) or blood-serum (RÖHMANN [1]) on starch. The formation of isomaltose in the hydrolysis of starch has been denied by many investigators because they considered isomaltose only as contaminated maltose.[2]

Isomaltose dissolves very readily in water, has a pronounced sweetish taste, and does not ferment, or, according to some, only very slowly. It is dextrorotatory, and has very nearly the same power of rotation as maltose. Isomaltose is characterized by its osazone. This forms fine yellow needles, which begin to form drops at 140° C. and melt at 150–153° C. These are rather easily soluble in hot water and dissolve in hot absolute alcohol much more readily than the maltosazone. Isomaltose reduces copper as well as bismuth solutions.

Lactose (MILK-SUGAR). As this sugar occurs exclusively in the animal world, in the milk of human beings and animals, it will be treated in a following chapter (on milk).

3. Colloid Polysaccharides.

If we exclude the not well known trisaccharides and the tetrasaccharide *stachyose* this group includes a great number of very complex carbohydrates which occur only in the amorphous condition, or at least not as crystals in the ordinary sense. Unlike the bodies belonging to the other groups, these have no sweet taste. Some are soluble in water, while others swell up therein, especially in warm water, and finally some are neither dissolved nor visibly changed. Polysaccharides are ultimately converted into monosaccharides by hydrolytic cleavage.

The polysaccharides are ordinarily divided into the following groups: *starches* with the *dextrins*, *plant gums* and *mucilages*, and the *celluloses*.

Starch Group.

Starch, AMYLUM $(C_6H_{10}O_5)x$. This substance occurs in the plant kingdom very extensively distributed in the different parts of the plant, especially as reserve food in the seed, roots, tubers, and trunks.

Starch is a white, odorless, and tasteless powder, consisting of small

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[1] Külz and Vogel, Zeitschr. f. Biologie, **31**; Röhmann, Centralbl. f. d. med. Wissensch., 1893, 849.

[2] Brown and Morris, Journ. of Chem. Soc., 1895; Chem. News, **72**. See also Ost Ulrich, and Jalowetz, Ref. in Ber. d. deutsch. chem. Gesellsch., **28**; Ling and Baker, Journ. of Chem. Soc., 1895; Pottevin, Chem. Centralbl., 1899, II, 1023.

granules which have a stratified structure and different shape and size in different plants. Starch is considered insoluble in cold water. The grains swell up in warm water and burst, yielding a paste.

According to the ordinary opinion the starch granules consist of two different substances, STARCH GRANULOSE and STARCH CELLULOSE (v. NÄGELI), the first of which turns blue with iodine and forms the chief part of the granule. According to MAQUENNE and ROUX [1] this is not the fact. According to them the starch granule consists of two constituents, of which the first, *amylose*, forms the chief mass (80-85 per cent) and the other, *amylopectin*, forms only 15–20 per cent of the granule. Amylopectin is not identical with v. NÄGELI's starch cellulose, and the above investigators consider starch cellulose as only an insoluble form of amylose. The amylose can occur in two forms; one, which is soluble, is colored blue by iodine and is immediately transformed into sugar by malt, the other is a solid substance, which is not colored with iodine and resists the action of malt infusion. One modification can be transformed into the other.

In the paste, besides amylopectin, we also have soluble amylose, and this can, by a process called *retrogradation* by MAQUENNE and ROUX, be transformed into the solid modification, "artificial starch." This solid form occurs in the starch granule, and is identical with v. NÄGELI's starch cellulose. As the starch granules are directly colored blue by iodine they must, besides this, also contain soluble amylose. If the author understands the above investigators correctly the starch granules contain three constituents, namely, soluble amylose, which is colored blue by iodine (=starch granulose), insoluble amylose, which is not colored by iodine (=starch cellulose), and amylopectin.

In the formation of paste the amount of amylose is not the essential but rather the quantity of amylopectin. The amylopectin is a slime-like substance, insoluble in boiling water and dilute alkalies, only becoming pasty therein, and not colored blue by iodine. Accordingly the paste is a solution of amylose made thick by amylopectin. The amylopectin, unlike the amylose, is only slowly transformed into sugar with dextrin formation. Starch is insoluble in alcohol and ether. On heating starch with water alone, or heating with glycerin to 190° C., or on treating the starch grains with 6 parts dilute hydrochloric acid of sp. gr. 1.06 at ordinary temperature for six to eight weeks,[2] it is converted into soluble starch (AMYLODEXTRIN, AMIDULIN). Soluble starch is also

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[1] v. Nägeli, Botan. Mitteil., 1863; Maquenne and Roux, Compt. rend., **138**, **140**, **142**, **146**, and Bull. Soc. chim. de Paris (3), **33** and **35**.

[2] See Tollens' Handb., 191. In regard to other methods, see Wróblewsky, Ber. d. deutsch. chem., Gesellsch., **30**; Syniewski, *ibid.*

formed as an intermediate step in the conversion of starch into sugar by dilute acids or diastatic enzymes. Soluble starch may be precipitated from very dilute solutions by baryta-water.[1]

Starch granules swell up and form a pasty mass in caustic potash or soda. This mass gives neither MOORE'S nor TROMMER'S test. Starch paste does not ferment with yeast. The most characteristic test for starch is the blue coloration produced by iodine in the presence of hydriodic acid or alkali iodides.[2] This blue coloration disappears on the addition of alcohol or alkalies, and also on warming, but reappears again on cooling.

On boiling with dilute acids starch is converted into glucose. In the conversion by means of diastatic enzymes we have, as a rule, besides dextrin, maltose, and isomaltose, only very little glucose. We are considerably in the dark as to the kind and number of intermediate products produced in this process (see Dextrins).

Starch may be detected by means of the microscope and by the iodine reaction. Starch is quantitatively estimated, according to SACHSSE'S method,[3] by converting it into glucose by hydrochloric acid and then determining the glucose by the ordinary methods.

Inulin $(C_6H_{10}O_5)x+H_2O$, occurs in the underground parts of many Compositæ, especially in the roots of the Inula helenium, the tubers of the Dahlia, the varieties of Helianthus, etc. It is ordinarily obtained from the tubers of the Dahlia.

Inulin forms a white powder similar to starch, consisting of spheroid crystals which are readily soluble in warm water without forming a paste. It separates slowly on cooling, but more rapidly on freezing. Its solutions are levogyrate and are precipitated by alcohol, and are colored only yellow with iodine. Inulin is converted into the levogyrate monosaccharide *d*-fructose on boiling with dilute sulphuric acid. Diastatic enzymes of higher animals have no, or only a very slight, action on inulin.[4]

According to DEAN[5] inulin occurs in combination with other substances, *levulins*, which are more soluble and have less rotation. He suggests that we limit the name inulin to that carbohydrate (or mixture of carbohydrates), which is readily precipitable by 60 per cent alcohol and shows a specific rotation of $(\alpha)_D = -38$ to 40°.

Lichenin (MOSS-STARCH) occurs in many lichens, especially in Iceland moss. It is not soluble in cold water, but swells up into a jelly. It is soluble in hot water, forming a jelly on allowing the concentrated solution to cool. It is colored yellow by iodine and yields glucose on boiling with dilute acids. Lichenin is not changed by diastatic enzymes such as ptyalin or amylopsin (NILSON[6]).

[1] In regard to the compounds of soluble starch and dextrins with barium hydroxide, see Bülow, Pflüger's Arch., **62**.

[2] See Mylius, Ber. d. deutsch. chem. Gesellsch., **20**, and Zeitsch. f. physiol. Chem., **11**.

[3] Tollens' Handb., 2. Aufl., **1**, 187.

[4] Tollens' Handbuch, 208.

[5] Amer. Chem. Journ., **32**.

[6] Upsala Läkaref. Förh., **28**.

Glycogen. This carbohydrate, which stands to a certain extent between starch and dextrin, is principally found in the animal kingdom, hence it will be considered in a subsequent chapter (on the liver).

Dextrins and Gums.

The dextrins stand in close relation to the starches, and are formed therefrom as intermediate products by the action of acids or diastatic enzymes. They yield as final products only hexoses, indeed only glucose, on complete hydrolysis. The vegetable gums, the vegetable mucilages and the pectin bodies, which all stand close to the hemicelluloses, yield, on the contrary, abundance of pentose and, among the hexoses, galactose is very often found.

Dextrin (starch-gum, British gum), is produced on heating starch to 200–210° C., or by heating starch, which has previously been moistened with water containing a little nitric acid, to 100–110° C. Dextrins are also produced by the action of dilute acids and diastatic enzymes on starch. There have been numerous investigations as to the steps involved in the last-mentioned process, but they have led to conflicting views. One of these, which used to be generally accepted, is as follows: The first product, which gives a blue color with iodine, is soluble starch or *amylodextrin*, which on further hydrolytic cleavage yields sugar and *erythrodextrin*, which is colored red by iodine. On further cleavage of this erythrodextrin more sugar and a dextrin, *achroodextrin*, which is not colored by iodine, is formed. From this achroodextrin after successive splittings we have sugar and dextrins of lower molecular weights formed, until finally we have sugar and a dextrin, *maltodextrin*, which refuses to split further, as final products. The views are rather contradictory in regard to the number of dextrins which occur as intermediate steps. The sugar formed is maltose (or in first place isomaltose), and only very little glucose is produced. Another view is that first several dextrins are formed consecutively in the successive splittings, by hydration, and then finally the sugar is formed by the splitting of the last dextrin. According to MOREAU, in the first stages of saccharification amylodextrin, erythrodextrin, achroodextrin and sugar are formed simultaneously. Other investigators, especially SYNIEWSKI, have recently suggested other views on the subject.[1]

This question has taken another direction by the investigations of

[1] In regard to the various views on the theories of the saccharification of starch, see Musculus and Gruber, Zeitschr. f. physiol. Chem., **2**; Lintner and Düll, Ber. d. d. chem. Gesellsch., **26** and **28**; Brown and Heron, Journ. of Chem. Soc., 1879; Brown and Morris, *ibid.*, 1885 and 1889; Moreau, Biochem. Centralbl., **3**, 648; Syniewski, Annal. d. Chem. u. Pharm., **309**, and Chem. Centralbl., 1902, **2**.

MAQUENNE, mentioned above. According to him the amylose passes directly into maltose without the formation of dextrin by the action of malt infusion. The dextrins produced are only formed from the amylopectin, which does not undergo saccharification with freshly prepared malt infusions, but only with older or especially active infusions. This also explains why in the older investigations the saccharification was only about 80 per cent while MAQUENNE has been able to completely convert the starch into sugar by enzymotic action.

The various dextrins are very hard to isolate as chemical individuals and to separate from each other. YOUNG[1] has tried their separation by means of neutral salts, especially ammonium sulphate, and MOREAU by the aid of a baryta-alcohol method. We cannot enter into the differences as to the dextrins so separated, and only the characteristic properties and reactions will be given for the dextrins in general.

The dextrins appear as amorphous, white or yellowish-white powders which are readily soluble in water. Their concentrated solutions are viscid and sticky, like gum solutions. The dextrins are dextrogyrate. They are insoluble or nearly so in alcohol, and insoluble in ether. Watery solutions of dextrins are not precipitated by basic lead acetate. Dextrins dissolve cupric hydroxide in alkaline liquids, forming a beautiful blue solution, which, as is generally admitted, is reduced by pure dextrins. According to MOREAU pure dextrin has no reducing action. The dextrins are not directly fermentable.

SCHARDINGER has discovered a bacillus which forms acetone from starch and which is especially useful for the perparation of crystalline cleavage products from starch. He obtained two crystalline substances, dextrin α and β, which are not fermentable by yeast and on hydrolysis with acid yield glucose. For the α-dextrin PRINGSHEIM and LANGHANS have determined the formula $(C_6H_{10}O_5)_4$ while BILTZ and TRUTHE[2] found the formula $(C_6H_{10}O_5)_6$ for the β-dextrin.

The **vegetable gums** are soluble in water, forming solutions which are viscid but may be filtered. We designate, on the contrary, as **vegetable mucilages** those varieties of gum which do not or only partly dissolve in water, and which swell up therein to a greater or less extent. The natural varieties of gum and mucilage, to which belong several generally known and important substances, such as gum arabic, wood-gum, cherry-gum, salep, and quince mucilage, and probably also the little-studied pectin substances, will not be treated in detail, because of their unimportance from a physiological standpoint.

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[1] Journ. of Physiol., **22**, which contains the older researches of Nasse, Krüger Neumeister, Pohl, and Halliburton. Moreau, l. c.

[2] Schardinger, Centralbl. f. Bak. u. Parasitenkunde, II, **22**, 98 (1909); **29**, 118 (1911); Pringsheim and Langhans, Ber. d. d. Chem. Gesellsch., **45**, 2533 (1912); Biltz and Truthe, *ibid.*, **46**, 1377 (1913).

The Cellulose Group $(C_6H_{10}O_5)x$.

Cellulose is that carbohydrate, or perhaps more correctly, mixture of carbohydrates, which forms the chief constituent of the walls of the plant-cells. This is true for at least the walls of the young cells, while in the walls of the older cells the cellulose is extensively incrusted with a substance called LIGNIN, and with many other cellulose derivatives and compounds.

The true celluloses are characterized by their great insolubility. They are insoluble in cold or hot water, alcohol, ether, dilute acids, and alkalies. We have only one specific solvent for cellulose, and that is an ammoniacal solution of copper oxide called SCHWEITZER'S reagent. The cellulose may be precipitated from this solvent by the addition of acids, and obtained as an amorphous powder after washing with water.

Cellulose is converted into a substance, so-called AMYLOID, which gives a blue coloration with iodine, by the action of concentrated sulphuric acid. With oxidizing agents (nitric acid, etc.) oxycelluloses are produced. By the action of strong nitric acid or a mixture of nitric acid and concentrated sulphuric acid, celluloses are converted into nitric-acid esters or nitrocelluloses, which are highly explosive and have found great practical use.

The ordinary celluloses when treated at the ordinary temperature with strong sulphuric acid and then boiled for some time after diluting with water are coverted into glucose. In this case it must be observed, according to MAQUENNE, that it is not maltose that is produced as an intermediate step, but another disaccharide, called *cellose* or *cellobiose.*

The cellulose, at least in part, undergoes decomposition in the intestinal tract of man and animals. A closer discussion of the nutritive value of cellulose will be given in a future chapter (on digestion). The great importance of the carbohydrates in the animal economy and to animal metabolism will also be given in the following chapters.

Hemicelluloses are, according to E. SCHULZE,[1] those constituents of the cell-wall related to cellulose which differ from the ordinary cellulose by dissolving on heating with strongly diluted mineral acids, such as 1.25 per cent sulphuric acid, and of yielding arabinose, xylose, galactose, and mannose instead of glucose. Those hemicelluloses which serve partly as reserve food and partly as support-substance, are very widely distributed in the plant kingdom. It must be recalled that according to BIERRY and GIAJA [2] the digestive organs of different invertebrates (Helix, Astacus, Maja. Hommarus) contain enzymes which have an energetic splitting action upon such polysaccharides as well as on the natural celluloses.

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[1] E. Schulze, Zeitschr. f. physiol. Chem., **16** and **19**, with Castro, *ibid.*, **86**.

[2] Bioch. Zeitschr., **40**, 370 (1912).

CHAPTER IV.

ANIMAL FATS AND PHOSPHATIDES.

1. Neutral Fats and Fatty Acids.

THE fats form the third chief group of the organic food of man and animals. They occur very widely distributed in the animal and plant kingdoms. Fat occurs in all organs and tissues of the animal organism, though the quantity may be so variable that a tabular exhibit of the amount of fat in different organs is of little interest. The marrow contains the largest quantity, having over 96 per cent. The three most important deposits of fat in the animal organism are the intermuscular connective tissue, the fatty tissue in the abdominal cavity, and the subcutaneous connective tissues. In plants, the seeds and fruit and in certain instances also the roots, are rich in fat. Fat also occurs deposited, during the winter's rest, in the trunks of trees.

The fats consist almost entirely of so-called neutral fats, with only very small quantities of fatty acids. The neutral fats are esters of the triatomic alcohol, glycerin, with monobasic fatty acids. These esters are triglycerides; that is, the hydrogen atoms of the three hydroxyl groups of the glycerin are replaced by the fatty-acid radicals, and their general formula is therefore, $C_3H_5.O_3.R_3$. The animal fats consist chiefly of esters of the three fatty acids, stearic, palmitic, and oleic acids. In certain fats, especially in milk-fat, glycerides of fatty acids such as butyric, caproic, caprylic, and capric acids also occur in considerable amounts. Besides the above-mentioned ordinary fatty acids, stearic, palmitic, and oleic acids, we also find in human and animal fat, exclusive of certain fatty acids only little studied, the following non-volatile fatty acids, as glycerides, namely, lauric acid, $C_{12}H_{24}O_2$, myristic acid, $C_{14}H_{28}O_2$, and arachidic acid, $C_{20}H_{40}O_2$. Of the unsaturated fatty acids, besides oleic acid, we probably also have in small quantities glycerides of acids of the linolic acid series $C_nH_{2n-4}O_2$ and of the linolenic acid series, $C_nH_{2n-6}.O_2$. In this case the question can be raised whether or not these acids are not derived from the phosphatides mixed with the fats. In the plant kingdom triglycerides of other fatty acids, such as lauric acid, myristic acid, linoleic acid, erucic acid, etc., sometimes occur

abundantly. Besides these, oxyacids and high molecular alcohols have been found in many plant fats. The extent to which traces of these oxyacids occur in the animal kingdom has not been thoroughly investigated, but the occurrence of monoxystearic acid seems to have been proved.[1] The occurrence of high molecular alcohols, although ordinarily only in small amounts, has on the contrary been positively shown in animal fat.

The animal fats are of the greatest interest and consist of a mixture of varying quantities of TRISTEARIN, TRIPALMITIN, and TRIOLEIN, having an average elementary composition of C 76.5, H 12.0, and O 11.5 per cent. It must be remarked that in animal fat (mutton and beef tallow) as well as in plant fat (olive-oil) mixed triglycerides, such as dipalmityl-olein, distearyl-palmitin and distearyl-olein, occur, and that these mixed glycerides may also be prepared synthetically.[2]

Fats from different species of animals, and even from different parts of the same animal, have an essentially different consistency, depending upon the relative amounts of the different individual fats present. In solid fats—as tallow—tristearin and tripalmitin are in excess, while the less solid fats are characterized by a greater abundance of triolein. This last-mentioned fat is found in greater quantities proportionally in cold-blooded animals, and this accounts for the fact that the fat of these animals remains fluid at temperatures at which the fat of warm-blooded animals solidifies. Human fat from different organs and tissues contains, in full numbers, 67-85 per cent triolein.[3] The melting-point of different fats depends upon the composition of the mixtures, and it not only varies for fat from different tissues of the same animal, but also for the fat from the same tissues in various kinds of animals.[4]

Neutral fats are colorless or yellowish, and, when perfectly pure, ordorless and tasteless. They are lighter than water, on which they float when in a molten condition. They are insoluble in water, dissolve in boiling alcohol, but separate on cooling—often in crystals. They are easily soluble in ether, benzene, chloroform, carbon disulphide and petroleum ether. The fluid neutral fats give an emulsion when shaken with a solution of gum or albumin. With water alone they give an emulsion

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[1] Erben, Zeitschr. f. physiol. Chem., **30**; Bernert, Arch. f. exp. Path. u. Pharm., **40**.

[2] Guth, Zeitschr. f. Biologie, **44**; W. Hansen, Arch. f. Hygiene, **42**; Holde and Stange, Ber. d. d. chem. Gesellsch., **34**; Kreis and Hafner, *ibid.*, **36**.

[3] See Knöpfelmacher, "Untersuch. über das Fett im Säuglingsalter," etc., Jarhbuch f. Kinderheilkunde (N. F.), **45**, which also contains the older literature; Jaeckle, Zeitschr. f. physiol. Chem., **36**.

[4] According to Gilkin (Ber. d. d. chem. Gesellsch., **41**) the fat from bone-marrow and also other fats of animal and plant origin contain iron, which cannot be removed by water containing hydrochloric acid.

only after vigorous and prolonged shaking, but the emulsion is not persistent. The presence of some soap causes a very fine and permanent emulsion to form easily. Fat produces spots on paper which do not disappear; it is not volatile; it boils at about 300° C. with partial decomposition, and burns with a luminous and smoky flame. The fatty acids have most of the above-mentioned properties in common with the neutral fats, but differ from them in being soluble in alcohol-ether, in having an acid reaction, and by not giving the acrolein test. The neutral fats generate a strong irritating vapor of acrolein, due to the decomposition of glycerin, $C_3H_5(OH)_3—2H_2O=C_2H_3.CHO$, when heated alone, or more easily when heated with potassium bisulphate or with other dehydrating substances.

The neutral fats may be split by the addition of the constituents of water according to the following equation:

$$C_3H_5(OR)_3+3H_2O=C_3H_5(OH)_3+3HOR.$$

This splitting may be produced by the pancreatic enzyme and other enzymes occurring in the animal and vegetable kingdoms, for example, the castor lipase. The reverse action, namely, the synthesis of fatty acid esters, can be brought about by enzymes, such as pancreatic lipase (see page 60). The cleavage of the neutral fats can also be accomplished by superheated steam or by dilute acids. We most frequently decompose the neutral fats by boiling them with not too concentrated caustic alkali, or, still better (in biochemical researches), with an alcoholic potash solution or with sodium alcoholate. By this procedure, which is called saponification, the alkali salts of the fatty acids (soaps) are formed. If the saponification is made with lead oxide, then lead plaster, the lead salt of the fatty acids is produced. By saponification is to be understood not only the cleavage of neutral fats by alkalies, but also the splitting of neutral fats into fatty acids and glycerin in general.

On keeping fats for a long time in contact with air they undergo a change, becoming yellow in color and acid in reaction, and they develop an unpleasant odor and taste, becoming *rancid*. In this change a part of the fat is split into fatty acids and glycerin, and then an oxidation of the free fatty acids takes place, producing volatile bodies of an unpleasant odor.

The three most important fats of the animal kingdom are *stearin*, *palmitin*, and *olein*.

Stearin, or tristearin, $C_{57}H_{110}O_6, = \begin{matrix} CH_2.O.C_{18}H_{35}O \\ CH.O.C_{18}H_{35}O \\ CH_2.O.C_{18}H_{35}O \end{matrix}$, occurs especially in the solid varieties of tallow but also in the vegetable fats. Stearic acid, $C_{18}H_{36}O_2$, is found in the free state in decomposed pus, in the expectora-

tions in gangrene of the lungs, and in cheesy tuberculous masses. It occurs as lime soap in excrement and adipocere, and in this last product also as an ammonium soap. It also exists as alkali soap in the blood, bile, transudations and pus, and in the urine to a slight extent.

Stearin is the hardest and most insoluble of the three ordinary neutral fats. It is nearly insoluble in cold alcohol, and soluble with great difficulty in cold ether (225 parts). It separates from warm alcohol on cooling as rectangular, and less frequently as rhombic plates. The opinions regarding the melting-point are somewhat varied. Pure stearin, according to HEINTZ,[1] melts transitorily at 55° and permanently at 71.5°. The stearin from the fatty tissues (not pure) melts at 63° C.

Stearic acid, $\begin{matrix}CH_3\\(CH_2)_{16}\\COOH\end{matrix}$, crystallizes (on cooling from boiling alcohol) in large, shining, long rhombic scales or plates. It is less soluble than the other fatty acids and melts at 68.2° C.[2] Its barium salt contains 19.49 per cent barium, and its silver salt contains 27.59 per cent silver.

Palmitin, or tripalmitin, $C_{51}H_{98}O_6, = \begin{matrix}CH_2.O.C_{16}H_{31}O\\CH.O.C_{16}H_{31}O\\CH_2.O.C_{16}H_{31}O\end{matrix}$. Of the two solid varieties of fats, palmitin is the one which occurs in predominant quantities in human fat (LANGER[3]). Palmitin is present in all animal fats and in several kinds of vegetable fat. A mixture of stearin and palmitin was formerly called MARGARIN. As to the occurrence of palmitic acid, $C_{16}H_{32}O_2$, about the same remarks apply as to stearic acid. The mixture of these two acids has been called margaric acid, and this mixture occurs —often as very long, thin, crystalline plates—in old pus, in expectorations from gangrene of the lungs, etc.

Palmitin crystallizes, on cooling from a warm saturated solution in ether or alcohol, in starry rosettes of fine needles. The mixture of palmitin and stearin, called margarin, crystallizes, on cooling from a solution, as balls or round masses which consist of short or long, thin plates or needles which often appear like blades of grass. Palmitin, like stearin, has a variable melting- and solidifying-point, depending upon the way it has been previously treated. The melting-point is often given as 62° C., but some investigators[4] claim that it melts at 50.5° C., solidifies on further heating, and melts again at 66.5° C.

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[1] Annal. d. Chem. u. Pharm., **92**.

[2] According to Carlinfanti and Levi-Malvano, Chem. Centralbl., 1910.

[3] Monatshefte f. Chem., **2**; see also Jaeckle, Zeitschr. f. physiol. Chem., **36**.

[4] R. Benedikt, Analyse der Fette, 3. Aufl., 1897, p. 44.

Palmitic acid, $\begin{matrix}CH_3\\(CH_2)_{14}\\COOH\end{matrix}$, crystallizes from an alcoholic solution in tufts of fine needles. It melts at 61° C.;[1] still the admixture with stearic acid, essentially changes them elting- and solidifying-points according to th relative amounts of the two acids. Palmitic acid is somewhat more soluble in cold alcohol than stearic acid; but they have about the same solubility in boiling alcohol, ether, chloroform, and benzene. Its barium salt contains 21.17 per cent barium, and silver salt contains 29.72 per cent silver.

Olein, or triolein, $C_{57}H_{104}O_6, = \begin{matrix}CH_2.O.C_{18}H_{33}O\\CH.O.C_{18}H_{33}O\\CH_2.O.C_{18}H_{33}O\end{matrix}$, is present in all animal fats, and in greater quantities in vegetable fats. It is a solvent for stearin and palmitin. The oleic acid (elaic acid), $C_{18}H_{34}O_2$, as soaps, probably has about the same occurrence as the other fatty acids.

Olein is, at ordinary temperatures, a nearly colorless oil of a specific gravity of 0.914, without odor or marked taste, and solidifies in crystalline needles at −6° C. It becomes rancid quickly if exposed to the air. It dissolves with difficulty in cold alcohol, but more easily in warm alcohol or in ether. It is converted into its isomer, ELAÏDIN, by nitrous acid.

Oleic acid, $\begin{matrix}CH_3\\(CH_2)_7\\CH\\\|\\CH\\(CH_2)_7\\COOH\end{matrix}$, is an unsaturated acid of the series $C_nH_{n-2}O_2$, and correspondingly takes up two halogen atoms, i.e., iodine, at the double bondage, a factor which is the basis of v. HÜBL'S method for determining the iodine equivalent. On taking up hydrogen, which can be accomplished by heating with hydroiodic acid and amorphous phosphorus, it is transformed into the corresponding saturated acid, namely, stearic acid. On oxidation the double bonds are satisfied by 2HO groups, and dioxystearic acid, $CH_3(CH_2)_7CHOH.CHOH(CH_2)_7COOH$, is formed. Oleic acid readily undergoes oxidation in the air with the formation of acid products, and the occurrence of monoxystearic acid, found in animal fats in certain instances, can be explained by this oxidation. Oleic acid on heating yields, besides volatile fatty acids, *sebacic acid*, $C_{10}H_{18}O_4$, which melts at 127°C; and with nitrous acid it is transformed into its isomer, solid *elaidic acid*, which melts at 45° C.

Oleic acid forms at ordinary temperature a colorless, tasteless, and

[1] Carlinfanti and Levi-Malvano. Chem. Centralbl. 1910.

odorless oily liquid which solidifies in crystals at about 4° C., which latter melt at 14° C. Oleic acid is insoluble in water, but dissolves in alcohol, ether, chloroform and petroleum ether. With concentrated sulphuric acid and some cane-sugar it gives a beautiful red or reddish-violet liquid whose color is similar to that produced in PETTENKOFER'S test for bile-acids. If a solution of oleic acid in glacial acetic acid is treated with a little chromic acid (in glacial acetic acid) and then with concentrated sulphuric acid, the green solution gradually becomes violet or cherry-red, and shows two characteristic absorption bands in the green, one a broad band near the blue and a second but fainter band near the yellow (LIFSCHÜTZ).[1] The barium salt of oleic acid contains 19.65 per cent barium and the silver salt 27.73 per cent silver.

If the watery solution of the alkali compounds of oleic acid is precipitated with lead acetate, a white, tough, sticky mass of lead oleate is obtained, which is not soluble in water and only slightly in alcohol, but is soluble in ether. This salt is more easily soluble in benzene than the lead salts of stearic and palmitic acids, and this behavior of the lead salts toward ether and benzene is made use of in separating oleic acid from the other fatty acids.

An acid related to oleic acid, DOEGLIC ACID, which is solid at 4° C., liquid at 16° C., and soluble in alcohol, is found in the blubber of the Balœna rostrata. According to BULL this acid is probably only a mixture of oleic acid and another acid—*gadoleic acid*, $C_{20}H_{38}O_2$, having a melting-point of +24.5° C., and occurring in cod-liver oil, herring oil and in whale blubber. In addition to this acid BULL found in cod-liver oil, besides myristic, palmitic, oleic and erucic acids, another acid, having the formula $C_{16}H_{30}O_2$. According to ELLMER[2] the most abundant acid (80–90 per cent) in cod-liver oil is *therapinic acid*, $C_{18}H_{28}O_2$ which is changed into stearic acid by reduction and *jecoleic acid*, which seems to be identical with BULL'S gadoleic acid. KURBATOFF has demonstrated the presence of linoleic acid in the fat of the silurus, sturgeon, seal, and certain other animals. Drying fats have also been found by AMTHOR and ZINK[3] in hares, wild rabbits, wild boar, and mountain-cock.

To detect the presence of fat in an animal fluid or tissue the fat must first be shaken out or extracted with ether. After the evaporation of the ether the residue is tested for fat and fatty acids. The neutral fats are differentiated from the fatty acids by the acrolein test, and the fatty acids by the fact that their solution in a mixture of alcohol and ether has an acid reaction. In separating the fats from cholesterin and other non-saponifiable substances, as well as for the determination of the kind of the various fatty bodies, they are saponified with caustic alkali, alcoholic potash, or with sodium alcoholate. In regard to these operations, as well as the further investigation and the separation of the

[1] Zeitschr. f. physiol. Chem., **56**.

[2] Bull, Ber. d. d. chem., Gesellsch., **39**; Ellmer, Bioch. Zeitschr., **9**.

[3] Kurbatoff, Maly's Jahresb., **22**; Amthor and Zink, Zeitschr. f. anal. Chem., **36**.

various fatty acids from each other, we must refer to more complete hand-books.

In addition to the methods already suggested there are other chemical methods which are important in investigating fats. Besides ascertaining the melting- and congealing-point we also determine the following: 1. The *acid equivalent*, which is a measure of the amount of fatty acids in a fat, is determined by titrating the fat dissolved in alcohol-ether with N/10 alcoholic caustic potash, using phenolphthalein as indicator. 2. The *saponification equivalent*, which gives the milligrams of caustic potash uniting with the fatty acids in the saponification of 1 gram fat with N/2 alcoholic caustic potash. 3. REICHERT-MEISSL'S *equivalent*, which gives the quantity of volatile fatty acids contained in a given amount of neutral fat (5 grams). The fat is saponified, then acidified with mineral acid, and distilled, whereby the volatile fatty acids pass over; the distillate is then titrated with alkali. 4. *Iodine equivalent* is the quantity of iodine absorbed by a certain amount of the fat by addition. It is chiefly a measure of the quantity of unsaturated fatty acids, principally oelic acid or olein, in the fat. Other bodies, such as cholesterin, may also absorb iodine or halogens. The iodine equivalent is generally determined according to the method suggested by V. HÜBL. 5. The *acetyl equivalent* measures the quantity of those constituents of fats which contain OH groups, and is found by converting these bodies (oxyfatty acids, alcohols and others) into the corresponding acetyl ester by boiling them with acetic acid anhydride.

In the quantitative estimation of fats, the finely divided dried tissues or the finely divided residue from an evaporated fluid is extracted with ether, alcohol-ether, benzene, or any other proper extraction medium. The lecithin (phosphatides) and other bodies are dissolved by the various extraction media, hence the results for fats are too high. The most exact method for the estimation of fat seems to be the method suggested by KUMAGAWA and SUTO,[1] who give a complete review of the literature of the subject.

The fats are poor in oxygen, but rich in carbon and hydrogen. They therefore represent a large amount of chemical energy, and yield correspondingly large quantities of heat on combustion. They take first rank among the foods in this regard, and are therefore of very great importance in animal life. We will speak more in detail of this significance, also of fat formation and of the behavior of the fats in the body, in the following chapters.

Cholesterin and isocholesterin ester, which will be discussed in a subsequent chapter, as well as the following bodies, are closely related to the fats.

Spermaceti. In the living spermaceti or white whale there is found, in a large cavity in the skull, an oily liquid called spermaceti, which on cooling, after death, separates into a solid crystalline part ordinarily called SPERMACETI, and into a liquid, SPERMACETI-OIL. This last is separated by pressure. Spermaceti is also found in other whales and in certain species of dolphin.

The purified, solid spermaceti, which is called CETIN, is a mixture of esters of fatty acids. The chief constituent is the cetyl-palmitic ester mixed with small

[1] Biochem. Zeitschr., 8. See also y. Schimidzu, *ibid.*, 28.

quantities of compound esters of lauric, myristic, and stearic acids with radicals of the alcohols, LETHAL, $C_{12}H_{25}.OH$, METHAL, $C_{14}H_{29}.OH$, and STETHAL, $C_{18}H_{37}.OH$.

Cetin is a snow-white mass shining like mother-of-pearl, crystallizing in plates, brittle, fatty to the touch, and which has a varying melting-point of 30 to 50° C., depending upon its purity. Cetin is insoluble in water, but dissolves easily in cold ether or volatile and fatty oils. It dissolves in boiling alcohol, but crystallizes on cooling. It is saponified with difficulty by a solution of caustic potash in water, but with an alcoholic solution it saponifies readily, and the above-mentioned alcohols are set free.

Ethal or cetyl alcohol, $C_{16}H_{34}O. = \begin{matrix} CH_3 \\ (\dot{C}H_2)_{14} \\ \dot{C}H_2.OH \end{matrix}$, which occurs in smaller quantities in beeswax, and was found by LUDWIG and v. ZEYNEK in the fat from dermoid cysts—though this is denied by AMESEDER,[1]—forms white, transparent, odorless, and tasteless crystals which are insoluble in water but dissolve easily in alcohol and ether. Ethal melts at 49.5° C.

SPERMACETI-OIL yields on saponification valeric acid, small amounts of solid fatty acids, and PHYSETOLEIC ACID. This acid, which has, like hypogæic acid, the composition $C_{16}H_{30}O_2$, occurs also, as found by LJUBARSKY,[2] in considerable amounts in the fat of the seal. It forms colorless and odorless needle-shaped crystals which easily dissolve in alcohol and ether and melt at 34° C.

BEESWAX may be treated here as concluding the subject of fats. It contains three chief constituents: (1) CEROTIC ACID, $C_{26}H_{52}O_2$,[3] which occurs as cetyl ether in Chinese wax and as free acid in ordinary wax. It dissolves in boiling alcohol and separates as crystals on cooling. The cooled alcoholic extract of wax contains (2) CEROLEIN, which is probably a mixture of several bodies, and (3) MYRICIN, which forms the chief constituent of that part of wax which is insoluble in warm or cold alcohol. Myricin consists chiefly of palmitic-acid ester of melissyl (myricyl) alcohol, $C_{30}H_{61}.OH$. This alcohol is a silky, shining, crystalline body melting at 85° C. DUNHAM [4] has found *carnaubic acid*, $C_{24}H_{48}O_2$, in a phosphatide from the ox kidney.

2. Phosphatides.

In close relation to the fats stands a group of esters containing nitrogen, phosphoric acid and fatty acid radicals. The representative of this group longest known is lecithin. This latter is an ester combination of a nitrogenous base, choline, with a fatty acid-glycerophosphoric acid, and THUDICHUM [5] has shown that a large number of more or less analogous bodies occur in the animal body, especially in the brain. All of these bodies have received the name *phosphatides*.

Those phosphatides which contain only one phosphoric acid radical in the molecule are called *monophosphatides;* those with two such radicals *diphosphatides*. The monophosphatides may contain one, two or more

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[1] Ludwig and v. Zeynek, Zeitschr. f. physiol. Chem. **23**; Ameseder, *ibid.*, **52**.

[2] Journ. f. prakt. Chem. (N. F.), **57**.

[3] See Henriques, Ber. d. deutsch. chem. Gesellsch., **30**, 1415.

[4] Journ. of biol. Chem., **4**.

[5] J. L. W. Thudichum, Die chemische Konstitution des Gehirns des Menschen, etc., Tübingen, 1901.

atoms of nitrogen in the molecule, and hence we differentiate between monamido- (P:N=1:1), diamido- (P:N=1:2), triamido- (P:N=1:3) monophosphatides, etc.

So also may the diphosphatides contain 1, 2 or 3 atoms of nitrogen for every 2 atoms of phosphorus (mono- di- or triaminodiphosphatides). Phosphatides with 4 or more atoms of nitrogen for every atom of phosphorus are also claimed to occur, but these statements seem to be uncertain. On the other hand, according to THUDICHUM, non-nitrogenous phosphatides occur in the brain; but if such be true these bodies must not, for the present at least, be classified as phosphatides.

The phosphatides thus far investigated seem to be chiefly ester combinations between nitrogenous bases and fatty acid-glycerophosphoric acid. According to THUDICHUM phosphatides exist which contain no glycerin group and the CARNAUBON obtained by DUNHAM[1] from beef kidneys seems to be such a phosphatide. The fatty acids occurring in the phosphatides may be of different kinds. It seems that at least one oleic acid radical, or another still less saturated fatty acid, occurs in most of the phosphatides; still we know of phosphatides that contain only saturated fatty acids. On this account the phosphatides may be divided into saturated and unsaturated phosphatides. The unsaturated add iodine, take up oxygen from the air and are auto-oxidizable and are changed readily. They also give a beautiful reaction with PETTENKOFER'S bile-acid test.

Choline has generally been obtained as a basic constituent of the phosphatides. Still other not sufficiently studied bases, have been found in the plant as well as animal phosphatides and according to TRIER[2] *aminoethyl alcohol* is a probable generally distributed component of the lecithins (phosphatides).

The phosphatides are very widely distributed in the plant as well as the animal kingdom and they must undoubtedly exist as primary cell constituents. We differentiate between such cell constituents which seem to be absolutely necessary for the life of the cells, and those which are stored up as reserve material, or are products of metabolism. The first, which seem to occur in all developing cells, have been called *primary* by KOSSEL[3] while he calls the others *secondary*. The question as to the division of the known cell constituents into the primary or secondary groups in the above sense, cannot be answered positively in many cases. In the primary group besides water and mineral bodies we include proteins of various kinds, nucleic acids and the so-called lipoids (see below) to which the phosphatides belong.

[1] Zeitschr. f. physiol. Chem., **64**, 303 (1910).
[2] *Ibid.*, **73**, **76** and **80**.
[3] Verhandl. d. physiol. Gesellsch. zu Berlin, 1890–91.

Attention has been called in Chapter I to the importance of the phosphatides (lipoids) for the limiting layer of the cells as well as for the osmotic processes and for the metabolism of the cells. The unsaturated, readily oxidizable phosphatides also play a possible rôle as oxygen carriers and the phosphatides are undoubtedly of great importance as constituents of the food-stuffs. There is also no doubt that they are very important for development and growth. It has been found that the amount of phosphatides is especially abundant in the new-born, and that these latter, to a certain extent, bring into the world a store of phosphatides and this store diminishes during growth.[1]

The phosphatides seem to be closely related to one another; they influence the solubility and precipitation properties of one another, and are generally precipitated as mixtures which are extremely difficult to separate into individual constituents. They are also amorphous, and readily oxidized, and it is easy to understand why their preparation in a pure state is so extremely difficult. Under these circumstances we have no sufficient guarantee as to their chemical individuality, and the description of their properties and composition must be accepted with some reservation.

The phosphatides are generally amorphous, colorless or yellowish; they melt, on warming, and burn. As a rule they are insoluble in water and swell up therein forming colloidal solutions, which are precipitated by certain salts. The phosphatides as above stated, belong to the lipoids and it is for this reason that each phosphatide is dissolved by at least one of the solvents for fats (alcohol, ether, benzene, petroleum ether, etc.). The lipoid group cannot be otherwise characterized. Originally we included in this group, bodies similar to fat or in certain respects related to the fats such as phosphatides, cholesterin and cerebrosides, but later the conception has been developed and now we consider as lipoids those bodies that are soluble in ether or equivalent. Under these circumstances, as the diverse known and unknown bodies, such as lactic acid, phenols, alkaloids and extractive bodies of various kinds may belong to the lipoid group there does not seem to be any sense in speaking of a special lipoid group, and especially from a chemical standpoint it would be better to drop the name entirely.

The various phosphatides show a different behavior toward the solvents for lipoids, namely some are soluble in ether while others are insoluble therein, etc., and these differences are important for their

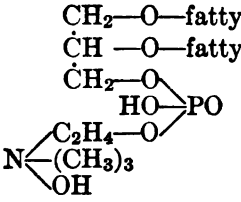
[1] In regard to the quantity and importance of the phosphatides (the lecithins) see Siwertzow, Bioch. Centralbl., **2**, 310; Glikin, Bioch. Zeitschr., **4** and **7**; Nerking, *ibid.*, **10**; Stoklasa, Ber. d. d. chem. Gesellsch., **29**, Wien. Sitz. Ber., **104**; Zeitschr. f. physiol. Chem. **25**; Danilewsky, Compt. Rend., **121** and **123** and W. Koch., Zeitschr. f. physiol. Chem., **37**; Kyes, *ibid.*, **41** and Berl. klin. Wochenschr., 1904.

preparation. They are generally all precipitated from their solution by acetone although not completely, and this behavior is also of especial importance in their preparation. The phosphatides are also nearly all precipitated by metallic salts, especially by platinum chloride and cadmium chloride, and this method is also often used in their preparation. The usefulness of this method has been questioned at least for certain phosphatides, since ERLANDSEN [1] showed that a decomposition occurs.

ERLANDSEN has also found that when finely divided heart-muscle, dried in the air, is completely extracted with ether and then with alcohol, the first extract contains the monophosphatides, and the alcohol extract contains the diamino phosphatides which were not free in the tissues, but existed in the combined state. Whether this observation is of general importance in the preparation of pure phosphatides remains to be seen.

As the phosphatides among themselves are rather difficult to characterize and as there is a question whether a pure phosphatide has thus far been prepared it seems of little interest to give a review here of the division of the isolated phosphatides among the different groups. In this chapter we will only discuss the three most studied phosphatides, namely, lecithin, cephalin and cuorin; the others will be treated of in the respective chapters.

Lecithins. In correspondence with the generally accepted view lecithin is a monoaminomonophosphatide, which forms an ester compound of glycerophosphoric acid substituted by two fatty-acid radicals with a base called choline,[2] hence there must exist several groups of lecithins. According to the kind of fatty acid contained in the lecithin molecule it is possible to have various lecithins, such as stearyl-, palmityl-, and oleyl-lecithins. According to THUDICHUM [3] every true lecithin always contains at least one oleic-acid radical. According to the investigations of HENRIQUES and HANSEN, COUSIN and ERLANDSEN,[4] there is no question that the so-called lecithin of the egg-yolk and muscles must contain a fatty acid, still less saturated than oleic acid. All lecithins are monaminophosphatides, according to the following type:



[1] Zeitschr. f. physiol. Chem., 51.

[2] Strecker, Annal. d. Chem. u. Pharm., 148; Hundeshagen, Journ. f. prakt. Chem. (N. F.), 28; Gilson, Zeitschr. f. physiol. Chem., 12. A different view is held by Malengreau and Pridgent, *ibid.*, 77.

[3] Thudichum, Die chemische Konstitution des Gehirns des Menschen, etc., Tübingen, 1901.

[4] Henriques and Hansen, Skand. Arch. f. Physiol., 14 (1903); Cousin, Compt. Rend., 137; Erlandsen, Zeitschr. f. physiol. Chem., 51.

The various lecithins stand close to each other in regard to constitution. The amount of phosphorus varies between 3.7–3.97 per cent and the amount of nitrogen between 1.7–1.9 per cent. The so-called di-stearyl-lecithin studied by HOPPE-SEYLER and DIACONOW,[1] which probably has a different structure, has the formula $C_{44}H_{90}NPO_9$. ERLANDSEN gives the formula $C_{43}H_{80}NPO_9$ for the lecithin isolated by him from the heart muscles.

On saponification with alkalies or baryta-water, lecithin yields fatty acids, glycerophosphoric acid, and choline. It is remarkable that in the cleavage of lecithins a smaller amount of nitrogen than corresponds to the choline is obtained. MAC LEAN[2] who has especially investigated this could not re-obtain the total nitrogen in the lecithins as choline but only a part thereof—from heart muscle lecithin, 42 per cent, and from egg-yolk lecithin, 65 per cent. He is therefore of the opinion that the choline group is not the only nitrogenous group in the lecithins and that therefore the generally accepted formula for lecithin is incorrect. TRIER[3] has indeed obtained aminoethyl alcohol as a cleavage product from several phosphatides, which he calls lecithins, but because of the difficulty in preparing phosphatides in a pure condition we are not sure that he was working with pure substances. Lecithin is slowly decomposed by dilute acids. Besides small quantities of glycerophosphoric acid we have large quantities of free phosphoric acid split off. The lecithins are also decomposed by enzymes (lipase) with the splitting off of fatty acids.

Lecithin is optically active, and as the glycerophosphoric acid which can be split off is also active, WILLSTÄTTER and LÜDECKE[4] claim that the phosphoric acid is not bound on the middle unsymmetric CH group, but rather at the end CH_2 group of glycerin.

Lecithin, according to HOPPE-SEYLER,[5] is found in nearly all animal and vegetable cells thus far studied, and also in nearly all animal fluids. It is especially abundant in the brain, nerves, fish eggs, yolk of the egg, electrical organs of the Torpedo electricus, semen, and pus, and also in the muscles and blood-corpuscles, blood-plasma, lymph, milk, especially woman's milk, and bile. Lecithin is also found in different pathological tissues or liquids. As the presence of lecithin is only indirectly determined by the detection of phosphorus in organic combinations, it must be borne in mind that the above assertions relate chiefly to the occurrence of phosphatides.

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[1] Hoppe-Seyler, Med. chem. Unters., Heft 2 and 3.
[2] Zeitschr. f. physiol. Chem., 59 and Bioch. Centralbl., 9.
[3] l. c., Zeitschr. f. physiol. Chem., 73, 76 and 80.
[4] Ber. d. d. chem. Gesellsch., 37.
[5] Physiol. Chem. Berlin, 1877–81, p. 57.

The same also applies to the claims as to the quantity of lecithin in various organs and tissues as well as in different ages. In these cases the lecithin has not been prepared in a pure state, and the determinations represent only the approximate quantity of phosphatides. These determinations of SIWERTZOW, GLIKIN and NERKING,[1] show that lecithins (phosphatides) occur abundantly in the bone marrow, suprarenal capsule, heart and lungs, besides in the spinal marrow, brain, and egg, and also that the quantity varies strikingly in different varieties of animals. NERKING found 41.7 per cent lecithin in the bone marrow and 21.33 per cent in the suprarenal capsule of the sea-urchin when calculated on the living organs, while the corresponding results in the rabbit were 2.71 and 2.39 per cent, respectively.

The statements as to the properties of the lecithins apply chiefly to the lecithin of the hen's egg, which since HOPPE-SEYLER and DIACONOW'S time has been considered as distearyl-lecithin without any positive foundation. Other lecithin preparations correspond essentially with this, and certain differences between the various lecithins may be possibly due to decomposition products or to admixture with other phosphatides. It is still questioned whether the so-called distearyl-lecithin is a unit body or not.

Lecithin may be obtained in grains or warty masses composed of small crystalline plates by thoroughly cooling its solution in strong alcohol. In the dry state it has a waxy appearance, is plastic, but forms pulverizable masses when dried in vacuum, and is soluble in alcohol, especially on heating (to 40–50° C.); it is less soluble in ether. It is dissolved also by chloroform, carbon disulphide, benzene, and fatty oils. The solution of lecithin from egg-yolk is dextrorotatory (ULPIANI[2]). P. MAYER[3] claims to have prepared racemic lecithin from ordinary lecithin, and *l*-lecithin from the *r*-lecithin by cleavage with lipase. As he did not make use of pure lecithin it is difficult to judge his results. The solution of lecithin in alcohol-ether or chloroform is precipitated by acetone, although not completely. It swells in water to a pasty mass which shows under the microscope slimy, oily drops and threads, so-called myelin forms (see Chapter XI). On warming this swollen mass or the concentrated alcoholic solution, decomposition takes place with the production of a brown color. On allowing the solution or the swollen mass to stand, decomposition takes place and the reaction becomes acid. According to the investigations of LONG[4] the lecithins seem to be much more

[1] Siwertzow, see Biochem. Zeitschr., **2**, p. 310; Glikin, Biochem. Zeitschr., **4** and **7**; Nerking, *ibid.*, **10**.

[2] Chem. Centralbl., 1901, **2**, 30 and 193.

[3] Biochem. Zeitschr., **1**.

[4] Journ. of Amer. chem. Soc., **30**, 1908.

resistant than was generally believed, and further investigations with pure lecithin are desirable.

With considerable water the lecithin gives an emulsion or colloidal solution which is not only precipitated by salts with divalent cations, Ca, Mg, and others as claimed by W. KOCH, but is also precipitated according to LONG and F. GEPHART[1] by salts with monovalent cations, although slowly. In putrefaction, lecithins yield glycerophosphoric acid and choline; the latter further decomposes with the formation of methylamine, ammonia, carbon dioxide, and marsh gas (HASEBROEK[2]). If dry lecithin be heated it decomposes, takes fire, and burns, leaving a phosphorized ash. On fusing with caustic alkali and saltpetre it yields alkali phosphates.

Lecithins combine with acids and bases. The compound with hydrochloric acid gives with platinum chloride a double salt which is insoluble in alcohol, soluble in ether, and which contains 10.2 per cent platinum (for distearyl-lecithin). The cadmium-chloride compound, whose composition has been found somewhat variable by different investigators is soluble with difficulty in alcohol, but dissolves in a mixture of carbon disulphide and ether or alcohol. A solution of lecithin in alcohol is not precipitated by lead acetate and ammonia.

Lecithins (and the same applies to the phosphatides in general) are easily carried down during the precipitation of other compounds, such as the protein bodies, and may therefore very greatly change the solubilities of other bodies. It is not known whether we are here dealing with an adsorption or a chemical combination, and the conditions are not the same in all cases. The combination with protein, the vitellines and lecithalbumins have been discussed in a previous chapter, and attention is there called to the necessity for more thorough investigation of this subject. Further investigations of the so-called lecithin-sugar (BING) is also desirable, as we know nothing definite as to its nature. According to the investigations of WINTERSTEIN, HIESTAND and E. SCHULZE, lecithins (phosphatides) containing carbohydrates occur in the plant kingdom, and contain about 20 per cent carbohydrate. We are still not decided whether we are here dealing with combinations or admixtures.[3] The same is true for the iron content of the lecithins or phosphatides as observed by GLIKIN.[4]

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[1] W. Koch., Zeitschr. f. physiol. Chem., **37**; Long and Gephart, Journ. of Amer. Chem. Soc., **30**; see also Porges and Neubauer, Biochem. Zeitschr., **7**.

[2] Zeitschr. f. physiol. Chem., **12**.

[3] Winterstein and Hiestand, Zeitschr. f. physiol. Chem., **47** and **54**; Schulze, *ibid.*, **52** and **55**; V. Njegovan, *ibid.*, **76**.

[4] Ber. d. d. chem. Gesellsch., **41**.

Various methods have been suggested by STRECKER, HOPPE-SEYLER and DIACONOW, THUDICHUM, GILSON, ZUELZER and BERGELL [1] for the preparation of the lecithins. As none of these yield a positively pure product we will here only mention them. According to ERLANDSEN'S experience all methods which are based upon the precipitation of the lecithin as a metallic compound should be avoided. The best method depends upon the solubility of the lecithin in alcohol and in ether in the cold and its precipitation by acetone (ERLANDSEN, H. E. ROAF and E. EDIE [2]). The work of ERLANDSEN is especially referred to in the preparation of lecithins.

For the present we have no quantitative method for estimating lecithin. The methods used in the past, when the amount of lecithin was calculated from the amount of phosphorus contained in the alcohol-ether extract is useless, as in this case the phosphorous content of all the phosphatides is determined and not alone of the lecithins. Even the detection of choline is not evidence, as this base probably occurs also in other phosphatides. In the detection of choline the double platinum compound is ordinarily prepared, and this can be done as described below. In special determinations of lecithin and cephalin KOCH used to heat with hydroiodic acid, and determined the methyl groups split off below 240° and those at about 300°. Instead of this he recommends with WOODS [3] to separate the two by precipitation in alcoholic solution, while boiling, with alcoholic lead acetate solution and a little ammonia, which precipitates only the cephalin.

Of the cleavage products of the lecithins choline is of especially great interest.

Choline (trimethyloxyethyl ammonium hydroxide),

$$C_5H_{15}NO_2, = HO.N\begin{cases}CH_2.CH_2.(OH)\\(CH_3)_3,\end{cases}$$

stands in close relation to the poisonous base *neurine* (trimethylvinyl ammonium hydroxide), $HO.N\begin{cases}(CH_3)_3\\CH{:}CH_2\end{cases}$, which according to BRIEGER can be formed from choline by the action of bacteria, and also to *muscarine*, $HO.N\begin{cases}(CH_3)_3\\CH_2CHO\end{cases}$, which is the aldehyde of choline and occurs in the fly agaric, and also to *betaine*, trimethyl glycocoll, $(CH_3)_3N\begin{cases}O\\CH_2\end{cases}CO$,

[1] Strecker, Annal. d. Chem. u. Pharm., **148**; Hoppe-Seyler and Diaconow, l. c.; Thudichum, l. c.; Gilson, Zeitschr. f. physiol. Chem., **12**; Zuelzer, *ibid.*, **27**; Bergell, Ber. d. d. chem. Gesellsch., **33**.

[2] Erlandsen. l. c.; Roaf and Edie, Thompson Yates Laboratory Reports, Vol. 6 part I, 1905.

[3] Koch and Woods, Journ. of biol. Chem., **1**.

which may be considered as the anhydride of the acid corresponding to choline. Muscarine and betaine can be obtained from choline on oxidation. Choline yields trimethylamine as a decomposition product, and this seems to be formed in the transformation of choline in the animal body.

Choline occurs in the plant kingdom as well as in the animal kingdom. MOTT and HALLIBURTON have repeatedly found choline in the blood in degenerative diseases of the nervous system. It was first shown also in normal blood by MARINO ZUCO,[1] and this investigator first found it in the suprarenal capsule, but designated it neurine. LOHMANN found it later in this organ, and recently it has been found in various organs by other investigators, especially by C. SCHWARZ and v. FÜRTH. The fact that choline is a cleavage product of lecithin in the animal, and that it is antagonistic to adrenalin (of the suprarenal capsule) by its depressing action upon the blood pressure, and that it has an exciting action upon certain secretions (LOHMANN, THEISSIER and THÉVENOT, v. FÜRTH and SCHWARZ[2]), gives choline great physiological importance. The physiological action of choline is still very much disputed.

Choline is a syrupy fluid, readily miscible with absolute alcohol. Hydrochloric acid gives with it a compound which is very soluble in water and alcohol, but insoluble in ether, chloroform, and benzene. This compound forms a double combination with platinum chloride, is soluble in water, insoluble in absolute alcohol and ether, and crystallizing from water in monoclinic system and this form is strongly double-refractive. From a mixture of water and alcohol it crystallizes in the regular form (octahedral). Both forms can be changed from one to the other and are used according to KAUFFMANN and VORLÄNDER[3] in the detection of choline. Choline also forms a crystalline double compound with mercuric chloride and with gold chloride. Choline is precipitated by potassium iodide and iodine (GULEWITSCH), and potassium triiodide can be used for the quantitative estimation of this base (STANEK[4]). On heating the free base it decomposes into trimethylamine, ethylene oxide, and water.

In preparing choline from lecithins, and also for the detection of lecithin in an alcohol-ether extract, proceed as follows: The residue from

[1] Mott and Halliburton, Philos. Trans., Ser. B, **191** (1899) and **194** (1901); Marino Zuco, see Maly's Jahresber., **24**, pp. 181 and 698.

[2] Lohmann, Pflüger's Arch., **118** and **122**; v. Fürth and Schwarz, *ibid.*, **124**, which also contains the literature.

[3] See Gulewitsch, Zeitschr. f. physiol. Chem., **24**; Kauffmann and Vorländer, Ber. d. d. chem. Gesellsch., **43**.

[4] Gulewitsch, Zeitschr. f. physiol. Chem., **24**; Stanek, *ibid.*, **56**. In regard to the quantitative estimation see also Kiesel, *ibid.*, **53**; Stanek, *ibid.*, **54**; Moruzzi, *ibid.*, **55**; and MacLean, *ibid.*, **55**.

the above, or the solid lecithin is boiled one hour with baryta-water, filtered, and the excess of baryta precipitated by CO_2; filter while hot, concentrate to a syrup, and extract with absolute alcohol, when the insoluble barium glycerophosphate remains; then precipitate the filtrate with an alcoholic platinum chloride solution.

Glycerophosphoric acid, $C_3H_9PO_6 = \begin{array}{l} CH_2.OH \\ \dot{C}H.OH \\ \dot{C}H_2—O \\ \quad OH—PO \\ \quad OH \end{array}$, is a bibasic acid which probably occurs in the animal fluids and tissues only as a cleavage product of lecithins. According to WILLSTÄTTER and LÜDECKE [1] the glycerophosphoric acid split off from lecithins is optically active. Its barium and potassium salts are levorotatory, and behave in certain respects differently from the corresponding salts of synthetically prepared glycerophosphoric acid. The Ba and Ca salts of glycerophosphoric acid are crystalline and are more soluble in cold than in warm water. The acid itself is a syrupy fluid.

Cephalin is also a monoaminophosphatide whose formula, based upon the investigations of THUDICHUM, KOCH, THIERFELDER and STERN,[2] is probably $C_{42}H_{82}NPO_{13}$. The views of these investigators as to the constitution of this body, which is difficult to purify, differ very considerably. According to THUDICHUM, on cleavage it yields neurine, glycerophosphoric acid, stearic acid, and a specific fatty acid, *cephalic acid.* According to KOCH it contains, on the contrary, only one methyl group attached to nitrogen, and is therefore probably dioxystearylmonomethyl lecithin. FRÄNKEL and DIMITZ found no choline, while according to COUSIN it yields, like lecithin, stearic acid, an unsaturated fatty acid, glycerophosphoric acid and choline as decomposition products. The glycerophosphoric acid from brain cephalin gives, according to FRÄNKEL and DIMITZ, a dextrorotatory Ba salt and is therefore not identical with the glycerophosphoric acid from lecithin. According to these investigators the cephalin of the human brain is a mixture of palmityl and stearylcephalin. Besides these two fatty acids cephalin also contains an unsaturated fatty acid, *cephalinic acid,* which according to PARNAS[3] is related to leinoleic acid or perhaps identical therewith.

From the investigations carried on thus far we can conclude that cephalin differs from lecithin in that it contains cephalinic acid, another glycerophosphoric acid and probably no choline but a monomethyl base. Cephalin has probably never been obtained in a pure form.

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[1] Ber d. d. chem. Gesellsch., 37.

[2] Thudichum, l. c.; Koch, Zeitschr. f. physiol. Chem., 36; Thierfelder and Stern, *ibid.* 53.

[3] Fränkel and Dimitz, Bioch. Zeitschr., 21; Parnas, *ibid.*, 22; Cousin, Compt. Rend. soc. biol., 62.

The cephalin from the brain has, according to FALK,[1] a different composition than that of the nerves and certain observations indicate that there are several cephalins.

Cephalin occurs quite abundantly in the brain and also in nerves and in the egg-yolk. The statements as to its further occurrence in the animal kingdom require substantiation.

Cephalin is amorphous, not very plastic, and more easily triturated than lecithin. It is readily soluble in cold ether, in chloroform and benzene but differs from lecithin by being insoluble or soluble with difficulty in alcohol. As unsaturated phosphatide it gives, like lecithin, a positive reaction with PETTENKOFER'S bile-acid test. The cadmium- and platinum chloride combinations are soluble in ether. Cephalin is obtained from the brain, after dehydration with acetone, by extracting with ether and precipitating the concentrated ethereal extract with alcohol. In regard to the preparation and detection of cephalin we must refer to more extensive hand-books.

The purest phosphatide prepared thus far seems to be cuorin, discovered by ERLANDSEN.

Cuorin, $C_{71}H_{125}NP_2O_{21}$, is a monaminodiphosphatide prepared by ERLANDSEN[2] from the heart muscle of the ox, and which has an iodine equivalent of 101. It yields as cleavage products 3 molecules fatty acids of unknown nature, partly or entirely belonging to the series $C_nH_{2n-4}O_2$ and $C_nH_{2n-6}O_2$; also glycerin, phosphoric acid and a base which is not well known, but it is not choline. Cuorin is autooxidizable, and gives PETTENKOFER'S bile-acid test.

Cuorin is amorphous, yellowish-brown and similar to rosin. It gives a neutral solution with water which is like an emulsion. Cuorin does not reduce FEHLING'S solution, even after boiling with acids. It is soluble in ether, chloroform, petroleum ether and carbon disulphide. It dissolves with difficulty in benzene; it is insoluble in ethyl and methyl alcohol and in acetone. Cuorin is precipitated from its alcohol-ether solution by cadmium or platinum chloride.

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[1] Bioch. Zeitschr., **18** and **16**.

[2] Zeitschr. f. physiol. Chem., **51**, where the method of preparation is described.

CHAPTER V.

THE BLOOD

THE blood is to be considered from a certain standpoint as a fluid tissue; it consists of a transparent liquid, the *blood-plasma*, in which a vast number of solid particles, the *red* and *white blood-corpuscles* (and the *blood-plates*), are suspended.

Outside of the organism the blood, as is well known, coagulates more or less quickly; but this coagulation is accomplished generally in a few minutes after leaving the body. All varieties of blood do not coagulate with the same degree of rapidity. Some coagulate more quickly, others more slowly. In vertebrates with nucleated blood-corpuscles (birds, reptiles, batrachia, and fishes) DELEZENNE has shown that the blood coagulates very slowly if it is collected under such precautions that it does not come in contact with the tissues. On contact with the tissues or with their extracts it coagulates in a few minutes. The blood with non-nucleated blood-corpuscles (mammals), on the contrary, coagulates very rapidly. The coagulation of the blood in these cases may also be somewhat retarded by preventing the blood from coming in contact with the tissues (SPANGARO, ARTHUS [1]). Among the varieties of blood of mammals thus far investigated the blood of the horse coagulates most slowly. The coagulation may be more or less retarded by quickly cooling; and if we allow equine blood to flow directly from the vein into a glass cylinder which is not too wide and which has been cooled, and let it stand at 0° C., the blood may be kept fluid for several days. An upper amber-yellow layer of plasma gradually separates from a lower red layer composed of blood-corpuscles with only a little plasma. Between these is observed a whitish-gray layer which consists of white blood-corpuscles.

The plasma thus obtained and filtered is a clear amber-yellow alkaline (toward litmus) liquid which remains fluid for some time when kept at 0° C., but soon coagulates at the ordinary temperature.

The coagulation of the blood may be prevented in other ways. After the injection of peptone, or, more correctly, proteose solutions into

[1] Delezenne, Compt. rend. soc. de biol., **49**; Spangaro, Arch. ital. de Biol., **32**; Arthus, Journ. de Physiol. et Pathol., **4**.

the blood (in the living dog), it does not coagulate on leaving the veins (FANO, SCHMIDT-MÜLHEIM [1]). The plasma obtained from such blood by means of centrifugal force is called *peptone-plasma*. According to ARTHUS and HUBER [2] the caseoses and gelatoses act like fibrin proteose in dogs. Eel serum and certain lymph-forming extracts of organs (see Chapter VI) have an analogous action. The coagulation of the blood of warm-blooded animals is prevented by the injection of an effusion of the mouth of the officinal leech or a solution of the active substance of such an infusion, *hirudin* (FRANZ), into the blood current (HAYCRAFT [3]). If the blood is allowed to flow directly, while stirring it, into a neutral salt solution—best a saturated magnesium-sulphate solution (1 vol. salt solution and 3 vols. blood)—we obtain a mixture of blood and salt which remains uncoagulated for several days. The blood-corpuscles, which, because of their adhesiveness and elasticity, would otherwise easily pass through the pores of the filter-paper, are made solid and stiff by the salt, so that they may be easily filtered off. The plasma thus obtained, which does not coagulate spontaneously, is called *salt-plasma*.

An especially good method of preventing coagulation of blood consists in drawing the blood into a dilute solution of potassium oxalate, so that the mixture contains 0.1 per cent oxalate (ARTHUS and PAGÈS [4]). The soluble calcium salts of the blood are precipitated by the oxalate, and hence the blood loses its coagulability. On the other hand, HORNE [5] found that chlorides of calcium, barium, and strontium, when present in large amounts (2–3 per cent), may prevent coagulation for several days. According to ARTHUS [6] a non-coagulable blood-plasma may be obtained by drawing the blood into a sodium-fluoride solution until it contains 0.3 per cent NaFl.

On coagulation there separates in the previously fluid blood an insoluble or a very difficultly soluble protein substance, *fibrin*. When this separation takes place without stirring, the blood coagulates in a solid mass, which, when carefully severed from the sides of the vessel, contracts, and a clear, generally yellow-colored liquid, the *blood-serum*, exudes. The solid coagulum which encloses the blood-corpuscles is called the *blood-clot* (placenta sanguinis). If the blood is beaten during coagulation, the fibrin separates in elastic threads or fibrous masses, and the

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[1] Fano, Arch f.. (anat. u.) Physiol., 1881; Schmidt-Mülheim, *ibid.*, 1880.

[2] Arch. de Physiol. (5), 8.

[3] Haycraft, Proc. Physiol. Soc., 1884, 13, and Arch. f. exp. Path. u. Pharm., 18; Franz, Arch. f. exp. Path. u. Pharm., 49.

[4] Archives de Physiol. (5), 2, and Compt. Rend., 112.

[5] Journ. of Physiol., 19.

[6] Journ. de Physiol. et Path., 3 and 4.

defibrinated blood which separates is sometimes called *cruor*[1] and consists of blood-corpuscles and blood-serum, while uncoagulated blood consists of blood-corpuscles and blood-plasma. The essential chemical difference between blood-serum and blood-plasma is that the blood-serum does not contain even traces of the mother-substance of fibrin, the fibrinogen, which exists in the blood-plasma, while the serum is proportionally richer in another body, the fibrin ferment (see below).

I. BLOOD-PLASMA AND BLOOD-SERUM.

The Blood-plasma.

In the coagulation of the blood a chemical transformation takes place in the plasma. A part of the proteins separate as insoluble fibrin. The albuminous bodies of the plasma must therefore be first described. They are, as far as we know at present, *fibrinogen*, *nucleoprotein*, *serglobulins*, and *seralbumins*.

Fibrinogen occurs in blood-plasma, chyle, lymph, certain transudates and exudates, in bone-marrow (P. MÜLLER), and perhaps also in other lymphoid organs. The seats of formation of fibrinogen are, according to MATHEWS, the leucocytes, especially of the intestine, according to MÜLLER, the bone-marrow and probably other lymphoid organs such as the spleen and lymph glands, and according to DOYON and NOLF, the liver. The statement that the intestinal wall is a seat of formation of fibrinogen, a view that had been held by DASTRE, is substantiated not only by the direct researches of MATHEWS, but also by the older and substantiated opinion that the blood from the mesentery vein is richer in fibrinogen than the arterial blood. This origin of fibrinogen has been shown to be improbable by the recent researches of DOYON, CL. GAUTIER and MOREL. The occurrence of fibrinogen in the bone-marrow and other lymphoid organs as shown by MÜLLER, and an increase of fibrinogen in the blood as well as in the bone-marrow of animals immunized with certain bacteria, especially pus-staphylococci, indicates the formation of fibrinogen in this tissue. The relation between the quantity of fibrin and leucocytosis as shown by many investigators such as LANGSTEIN and MAYER, MORAWITZ and REHN, also indicate such a formation of fibrinogen. The observations of DOYON, GAUTIER and MAWAS that a rapid re-formation of fibrinogen takes place in splenectomized animals

[1] The name *cruor* is used in different senses. We sometimes mean thereby only the blood when coagulated in a red solid mass, in other cases the blood-clot after the separation of the serum, and again the sediment consisting of red blood-corpuscles which is obtained from defibrinated blood by means of centrifugal force or by letting it stand.

without any changes in the bone-marrow speak against the especially great importance of the spleen and bone-marrow for the formation of fibrinogen. That the liver takes part in the formation of fibrinogen is implied by the fact that the quantity of fibrinogen in the blood strongly diminishes after the extirpation of the liver (NOLF), and that fibrinogen may indeed be entirely absent in the blood in phosphorus poisoning (CORIN and ANSIAUX, JACOBY, DOYON, MOREL, and KAREFF [1]), and that the blood of the hepatic vein, according to DOYON, MOREL and KAREFF, is richer in fibrinogen than the blood from other vessels, and finally according to WHIPPLE and HURWITZ [2] in chloroform poisoning the fibrinogen content of the blood diminishes with the injury to the liver and rises again with restitution of the organ.

Fibrinogen has the general properties of the globulins, but differs from other globulins as follows: In a moist condition it forms white flakes which are soluble in dilute common salt solutions, and which easily conglomerate into tough, elastic masses or lumps. The solution in 5–10 per cent NaCl coagulates on heating at 52–55° C., and the faintly alkaline or nearly neutral weak salt solution coagulates at 56° C., or at exactly the same temperature at which the blood-plasma coagulates. Fibrinogen solutions are precipitated by an equal volume of a saturated common salt solution, and are completely precipitated by adding an excess of NaCl in substance (thus differing from serglobulin). A salt-free solution of fibrinogen in as little alkali as possible gives with $CaCl_2$ a precipitate which contains calcium and soon becomes insoluble. In the presence of NaCl or by the addition of an excess of $CaCl_2$ the precipitate does not appear.[3] A neutral solution of fibrinogen is precipitated by a concentrated solution of sodium fluoride when added in a sufficient quantity. Fibrinogens from different kinds of blood behave somewhat differently in this regard. According to HUISKAMP [4] fibrinogen from horse-blood hardly dissolves in NaCl of 3–5 per cent at ordinary temperatures, while it does dissolve at 40–45°. It also dissolves in ammonia of 0.05

[1] P. Müller, Hofmeister's Beiträge, **6**; Mathews, Amer. Journ. of Physiol., **3**; Nolf, Bull. Acad., Roy. Belg., 1905, and Arch. intern. de Physiol., **3**, 1905; Langstein, and Mayer, Hofmeister's Beiträge, **5**; Morawitz and Rehn, Arch. f. exp. Path. u. Pharm., **58**; Corin and Ansiaux, Maly's Jahresber., **24**; Jacoby, Zeitschr. f. physiol. Chem., **30**; Doyon, Morel and Kareff, Compt. Rend., **140**; Doyon, Morel, and Péju, Comp. rend. soc. biolog., **58**; Doyon, Cl. Gautier, and Morel *ibid.*, **62**; Doyon, Gautier and Mawas, *ibid.*, **64**.

[2] Doyon, Morel and Kareff, Journ. de Physiol., 8 (1906); Whipple and Hurwitz, Journ. of exp. Med. **13**. See also Meek, Amer. Journ. of Physiol., **30**.

[3] See Hammarsten, Zeitschr. f. physiol. Chem., **22**; Cramer, *ibid.*, **23**.

[4] Huiskamp, *ibid.*, **44** and **46**. In regard to fibrinogen the reader is referred to the author's investigations. Pflüger's Archiv., **19** and **22**, and Zeitschr. f. physiol. Chem., **28**.

per cent, and on the addition of 3–5 per cent NaCl this solution can be neutralized. The fibrinogen prepared by HUISKAMP in this way retained its typical properties. Fibrinogen differs from the myosin of the muscles, which coagulates at about the same temperature, and from other protein bodies, in the property of being converted into fibrin under certain conditions. Fibrinogen has a strong decomposing action on hydrogen peroxide. It is quickly made insoluble by precipitation with water or with dilute acids. Its specific rotation is $(\alpha)_D = -52.5°$ according to MITTELBACH.[1]

Fibrinogen may be easily separated from the salt-plasma or oxalate-plasma by precipitation with an equal volume of a saturated NaCl solution. It must be observed that the oxalate-plasma can only be employed after the precipitate, containing proenzymes, and produced by exposure to cold, has settled and been filtered off. If this is not done then the fibrinogen is always impure. For further purification the precipitate is pressed, redissolved in an 8-per cent salt solution, the filtrate precipitated by a saturated salt solution as above, and after being treated in this way three times, the precipitate at last obtained is pressed between filter-paper and finely divided in water. The fibrinogen dissolves with the aid of the small amount of NaCl contained in itself, and the solution may be made salt-free by dialysing with very faintly alkaline water. The fibrinogen can be almost freed from fibrin-globulin, which will be spoken of later, by precipitating with double the volume of saturated sodium-fluoride solution, redissolving in water with 0.05-per cent ammonia, and then neutralizing this solution, treated with NaCl, and repeating this several times. Fibrinogen may also, according to REYE,[2] be prepared by fractionally precipitating the plasma with a saturated solution of ammonium sulphate. We have no knowledge as to the purity of the fibrinogen so prepared. The methods for the detection and quantitative estimation of fibrinogen in a liquid were formerly based on its property of yielding fibrin on the addition of a little blood, of serum, or of fibrin ferment. REYE has suggested the fractional precipitation with ammonium sulphate as a quantitative method. The value of this method has not been sufficiently tested.

Fibrinogen stands in close relation to its transformation product, fibrin.

Fibrin is the name of that protein body which separates on the so-called spontaneous coagulation of blood, lymph, and transudates as well as in the coagulation of a fibrinogen solution after the addition of serum or fibrin ferment (see below).

If the blood is beaten during coagulation, the fibrin separates in elastic, fibrous masses. The fibrin of the blood-clot may be beaten to

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[1] Zeitschr. f. physiol. Chem., **19**.

[2] W. Reye, Ueber Nachweis und Bestimmung des Fibrinogens, Inaug-Diss. Strassburg, 1898.

small, less elastic, and not particularly fibrous, lumps. The typical fibrous and elastic white fibrin, after washing, stands, in regard to its solubility, close to the coagulated proteins. It is insoluble in water, alcohol, or ether. It expands in hydrochloric acid of 1 p. m., as also in caustic potash or soda of 1 p. m., to a gelatinous mass, which dissolves at the ordinary temperature only after several days; but at the temperature of the body it dissolves more readily, although still slowly. Fibrin may be dissolved by dilute salt solutions, after a long time, at the ordinary temperature, or much more readily at 40° C., and this solution takes place, according to ARTHUS and HUBERT and also DASTRE,[1] without the aid of micro-organisms. This action is due to proteolytic enzymes carried down by the fibrin or enclosed within the leucocytes, (RULOT[2]). According to GREEN and DASTRE[3] two globulins are formed in the solution of fibrin in neutral salt solution, and according to RULOT also proteoses (and peptones) on the solution of fibrin containing leucocytes. Fibrin, like fibrinogen, decomposes hydrogen peroxide, due to a contamination with catalases, but this property is destroyed by heating or by the action of alcohol.

What has been said of the solubility of fibrin relates only to the typical fibrin obtained from the arterial blood of oxen or man by whipping and washing first with water and with common salt solution, and then with water again. The blood of various kinds of animals yields fibrin with somewhat different properties, and according to FERMI[4] pig-fibrin dissolves much more readily than ox-fibrin in hydrochloric acid of 5 p. m. Fibrins of varying purity or originating from blood from different parts of the body have unlike solubilities.

The fibrin obtained by beating the blood, and purified as above described, is always contaminated by secluded blood-corpuscles or remains thereof, and also by lymphoid cells. It can be obtained pure only from filtered plasma or filtered transudates. For the preparation of pure fibrin, as well as for the quantitative estimation of it, the spontaneously coagulating liquid is at once, or the non-spontaneously coagulating liquid only after the addition of blood-serum or fibrin ferment, thoroughly beaten with a whale-bone, and the separated coagulum is washed first in water and then with a 5-per cent common salt solution, and again with water, and finally extracted with alcohol and ether. If the fibrin is allowed to stand for some time in contact with the blood from which it was formed, it partly dissolves (*fibrinolysis*—DASTRE[5]). This fibrinolysis must be prevented in the exact quantitative estimation

[1] Arthus and Hubert, Arch. de Physiol. (5) 5; Dastre, *ibid.*, (5) 7.
[2] Arch. intern. de Physiol., 1.
[3] Green, Journ. of Physiol., 8; Dastre, l. c.
[4] Zeitschr. f. Biologie, 28.
[5] Archives, de Physiol. (5), 5 and 6.

of fibrin (DASTRE). The blood constituents that are active in fibrinolysis are still unknown, but they are without doubt of enzymotic nature. It must be mentioned that a strong fibrinolysis takes place in blood after acute phosphorus poisoning (JACOBY and others), after extirpation of the liver (NOLF), and also when the coagulability of the blood has been reduced by the injection of proteoses (NOLF, RULOT [1]).

A pure fibrinogen solution may be kept at the ordinary temperature until putrefaction begins without showing a trace of fibrin coagulation. But if to this solution is added a water-washed fibrin-clot or a little blood-serum, it immediately coagulates, and may yield a perfect typical fibrin. The transformation of the fibrinogen into fibrin requires the presence of another body contained in the blood-clot and in the serum. This body, whose importance in the coagulation of fibrin was first observed by BUCHANAN [2], was later rediscovered by ALEXANDER SCHMIDT,[3] and designated as *fibrin ferment* or *thrombin*. The nature of this enzymotic body has not been ascertained with certainty. Even after careful purification it gives very faint protein reactions and it is a much disputed question whether it is a globulin or a nucleoprotein. It is a fact that powerfully active solutions of thrombin can be obtained that do not give either the reactions for globulins or nucleoproteins. Fibrin ferment is produced, according to PEKELHARING,[4] by the influence of soluble calcium salts on a preformed zymogen existing in the non-coagulated plasma. SCHMIDT admits the presence of such a mother-substance of the fibrin ferment in the blood, and calls it *prothrombin*. The conversion of this mother-substance into thrombin is a very complicated process, which will be discussed under the coagulation of the blood. Thrombin behaves like other enzymes in that the very smallest amount of it produces an action, and its solution becomes inactive on heating. The velocity of coagulation is dependent upon the quantity of thrombin, and indeed a time law has been proposed for the action of thrombin. According to FULD the action of thrombin, at least within certain limits, follows SCHÜTZ'S law, and according to STROMBERG the thrombin follows in its action a time law, which at least in the beginning, corresponds to

[1] Jacoby, Zeitschr. f. physiol. Chem., **30**; Nolf, Arch. intern. de Physiol., **3**, 1905; Rulot, l. c.

[2] London Med. Gazette, 1845, 617. Cit. by Gamgee, Journal of Physiol., 1879.

[3] Pflüger's Arch., **6**; see also Zur Blutlehre, 1892, and Weitere Beiträge zur Blutlehre, 1895.

[4] Pekelharing, Verhandl. d. Kon. Akad. d. Wetensch. te Amsterdam, 1892, Deel 1; *ibid.*, 1895, and Centralbl. f. Physiol., **9**; Wright, Proc. Roy. Irish Acad. (3), **2**; The Lancet, 1892, and On Wooldridge's Method, etc., British Med. Journal, 1891; Lilienfeld, Hämatol. Untersuch. Arch. f. (Anat. u.) Physiol., 1892; Ueber Leukocyten und Blutgerinnung, *ibid.*; Halliburton and Brodie, Journal of Physiol., **17** and **18**; Huiskamp, Zeitschr. f. physiol. Chem., **32**; Pekelharing and Huiskamp, *ibid.*, **39**.

SCHÜTZ's law while on increasing dilution deviates more and more and finally shows a proportionally slow, and more irregular procedure. MARTIN [1] has found another law from experiments with plasma and snake-poisons containing thrombin. According to him the behavior is as follows: As in the casein coagulation with rennin, the celerity of coagulation is inversely proportional to the quantity of ferment; and LOEB has observed a similar conduct with invertebrates. The optimum of the thrombin action lies at about 40° C.; at 70–75° C., in neutral solution, the enzyme is destroyed. According to HOWELL and RETTGER [2] thrombin, under proper conditions, can withstand boiling for a short time. The question as to whether the thrombin found in different animals is the same substance or whether we have several thrombins, has not been decided. The latter is not improbable; nevertheless a definite specificity of different thrombins has not been observed with certainty.

The isolation of thrombin has been tried in several ways. Ordinarily, it may be prepared by the following method, proposed by ALEX. SCHMIDT: Precipitate the serum or defibrinated blood with 15–20 vols. of alcohol and allow it to stand a few months. The precipitate is then filtered off and dried over sulphuric acid. The ferment may be extracted from the dried powder by means of water. Other methods have been suggested by HAMMARSTEN, PEKELHARING, and HOWELL.[3] According to a method suggested by HAMMARSTEN a solution of thrombin so poor in lime salts that it contains only 0.3–0.4 p. m. solids and about 0.0007 p. m. CaO can be prepared.

If a fibrinogen solution containing salt, as above prepared, is treated with a solution of thrombin, it coagulates at the ordinary temperature more or less quickly and yields a typical fibrin. Besides the thrombin, the presence of neutral salts is necessary, for ALEX. SCHMIDT has shown that fibrin coagulation does not take place without them. The presence of soluble calcium salts is not, as is generally assumed, a positive condition for the formation of fibrin, because, thrombin can transform fibrinogen into typical fibrin in the absence of lime salts precipitable by oxalate.[4] The fibrin is not richer in lime than the fibrinogen used in its preparation if the fibrinogen and thrombin solutions are employed as lime-free as possible, and the view that the fibrin formation is connected with a taking up of lime has been shown to be untenable (HAMMARSTEN). The quantity of fibrin obtained on coagulation is always smaller than

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[1] Martin, Journ. of Physiol., **32**; Fuld, Hofmeister's Beiträge, **2**; Loeb, *ibid.*, **9**; Stromberg, Biochem. Zeitschr., **37**.

[2] Howell, Amer. Journ. of Physiol., **26**; Rettger, *ibid.*, **24**.

[3] Hammarsten, *ibid.*, 18; Pekelharing, l. c.; Howell, l. c.

[4] See Hammarsten, Zeitschr. f. physiol. Chem., **22**, which also cites the works of Schmidt and Pekelharing, and *ibid.*, **28**.

the amount of fibrinogen from which the fibrin is derived, and we always find a small amount of protein substance in the solution. It is therefore not improbable that the fibrin coagulation, in accordance with the views first proposed by DENIS, is a cleavage process in which the soluble fibrinogen is split into an insoluble protein, the fibrin, which forms the chief mass, and a soluble protein substance which is produced only in small amounts. We find a globulin-like substance which coagulates at about 64° C. in blood-serum as well as in the serum from coagulated fibrinogen solutions. This substance is called *fibrin-globulin* by HAMMARSTEN. The investigations of HUISKAMP have shown that this substance is not formed as a cleavage product from pure fibrinogen, but occurs in plasma or in fibrinogen solutions not purified from sodium fluoride or perhaps in loose combination with fibrinogen. The view that a cleavage takes place in the coagulation of the fibrinogen has not been supported by these investigations.[1]

Opinions are not unanimous in regard to the enzyme nature of thrombin and the enzymotic formation of fibrin, and there are, indeed, investigators who consider the coagulation as another process. A more thorough discussion of this subject can take place only in connection with the coagulation of the blood.

Nucleoprotein. This substance, which, as above-mentioned, is considered by PEKELHARING and HUISKAMP as identical with the prothrombin or thrombin, occurs in the blood-plasma as well as in the serum, and is precipitated from the latter with the globulin. It is similar to the globulin in that it is readily soluble in neutral salt solution, and can be completely salted out on saturation with magnesium sulphate, and separates only incompletely on dialysis. It is much less soluble than serglobulin in an excess of dilute acetic acid, and coagulates at 65–69° C. C. G. LIEBERMEISTER [2] found only 0.08–0.09 per cent phosphorus in the nucleoprotein, which indicates that the nucleoprotein was contaminated with other proteins. He also found that the substance was soluble in acetic acid with difficulty, a property which is used by PEKELHARING as an important means of separating the compound proteins from the globulins.

Serglobulins, also called *paraglobulin* (KÜHNE), *fibrinoplastic substance* (ALEX. SCHMIDT), *serum-casein* (PANUM [3]), occur in the plasma, serum, lymph, transudates and exudates, in the white and red corpuscles, and probably in many animal tissues and form-elements, though in small quantities. They are also found in the urine in many diseases.

The so-called serglobulin is without doubt not an individual substance, but consists of a mixture of two or more protein bodies which

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[1] See Hammarsten, Zeitschr. f. physiol. Chem., 28; Heubner, Arch. f. exp. Path. u. Pharm., 49, and Zeitschr. f. physiol. Chem., 45; Huiskamp, *ibid.*, 44 and 46.

[2] Hofmeister's Beiträge, 8; Pekelharing and Huiskamp, l. c. footnote 1, page 256.

[3] Kühne, Lehrbuch d. physiol. Chem., Leipzig., 1866–68; Alex. Schmidt, Arch. f. (Anat. u.) Physiol., 1861–62; Panum, Virchow's Arch., 3 and 4.

cannot be completely and positively separated from each other. The mixture of globulins obtained from blood-plasma or blood-serum by saturation with magnesium sulphate or half-saturation with ammonium sulphate consists of nucleoprotein, fibrin-globulin, and the true serglobulin or mixture of globulins.

The nucleoprotein has been previously discussed. The fibrin-globulin, which occurs in the serum only in small amounts, can be completely precipitated by NaCl. It has the general properties of the globulins, but differs from the serglobulins by a lower coagulation temperature, 64–66° C., and also in that it is precipitated by $(NH_4)_2SO_4$ even in 28 per cent solution.

Serglobulins. If the globulin obtained by saturation with magnesium sulphate is dialyzed, then, as has been known for a long time and further substantiated by MARCUS, only a part of the globulin separates out, while a portion remains in solution and cannot be precipitated by the addition of acid. For this reason MARCUS [1] also differentiates between a water-soluble globulin and one insoluble in water. According to the later investigations of HOFMEISTER and PICK [2] the part insoluble in water corresponds chiefly to a globulin fraction readily precipitated by $(NH_4)_2SO_4$ (by 28–36 vols. per cent saturated solution), and the part soluble in water corresponds to a fraction difficult to precipitate (by 36–44 vols. per cent saturated solution). The first fraction is called *euglobulin* and the second *pseudoglobulin*. According to PORGES and SPIRO [3] the serglobulins can be separated by $(NH_4)_2SO_4$ into three fractions whose precipitation limits are 28–36, 33–42, and 40–46 vols. per cent saturated solution. All three fractions contain globulin insoluble in water. FREUND and JOACHIM [4] have found that the euglobulin as well as the pseudoglobulin fraction is a mixture of globulin soluble in water and globulin insoluble in water, and consequently the number of different globulins in the serum may be still greater.

It follows from all these investigations that either the difference between the globulin soluble in water and that insoluble is not sufficient or that the fractional precipitation with ammonium sulphate is not suited for the separation of the various globulins. This latter seems to be the case, as shown by MELLANBY HASLAM and recently by WIENER.[5] It must not be forgotten that the globulin fractions are always contaminated with other serum constituents, and that these may influence the solubility and precipitability. As HAMMARSTEN has shown, a water-soluble globulin, can be transformed into a globulin insoluble in water

[1] Zeitschr. f. physiol. Chem., 28.

[2] Hofmeister's Beiträge, 1.

[3] Hofmeister's Beiträge, 8.

[4] Zeitschr. f. physiol. Chem., 36.

[5] Mellanby, Journ. of Physiol., 36; Haslam, *ibid.*, 32; Wiener, Zeitschr. f. physiol Chem., 74.

by careful purification, and also the reverse, namely, a globulin insoluble in water can sometimes be converted into one soluble in water by allowing it to lie in the air. An insoluble protein like casein can also, according to HAMMARSTEN,[1] have the solubilities of a globulin due to contamination with constituents of the serum, and K. MÖRNER [2] has also shown that a contamination of the serum-globulins with soap can essentially modify the precipitation of these globulins. Under these circumstances the above assumptions in regard to the different globulin fractions must be accepted with great caution.

The investigations made thus far upon the so-called serglobulin, have not led to any positive results. That this globulin, with the exception of the enzymes, antienzymes, immune bodies, and other unknown substances which are carried down by the various fractions, is a mixture of globulins there seems to be no doubt. The serglobulin or the globulin mixture which is obtained from the serum by the methods to be described has the following properties:

In a moist condition it forms snow-white flaky masses, neither tough nor elastic, which always contain thrombin and hence can bring about coagulation in a fibrinogen solution. The neutral solution is only incompletely precipitated by NaCl added to saturation, and is not precipitated by an equal volume of a saturated salt solution. It is only partly precipitated by dialysis or by the addition of acid. On saturation with magnesium sulphate, or one-half saturation with ammonium sulphate a complete precipitation is obtained. The coagulation temperature is, with 5–10 per cent NaCl in solution, 69–76°, but more often 75° C. The specific rotation of the solution containing salt is $(\alpha)_D = -47.8°$ for the serglobulin from ox-blood (FREDERICQ [3]). The various globulin fractions do not differ essentially from each other in their coagulation temperatures, specific rotation, refraction coefficient (REISS [4]), and their elementary composition. The average composition is, according to HAMMARSTEN, C. 52.71, H 7.01, N 15.85, S 1.11 per cent. K. MÖRNER [5] found 1.02 per cent sulphur and 0.67 per cent lead-blackening sulphur. All the sulphur seems to exist as cystine.

Serglobulin contains, as K. MÖRNER first showed, a carbohydrate group which can be split off. LANGSTEIN [6] has obtained several carbohydrates from the blood-globulin, namely, glucose, glucosamine,

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[1] See Hammarsten, Ergebnisse, d. Physiol., **1**, Abt. 1.

[2] Zeitschr. f. physiol. Chem., **34**.

[3] Bull. Acad. Roy. de Belg. (2), **50**. In regard to paraglobulin, see Hammarsten, Pflüger's Arch., **17** and **18**, and Ergebnisse d. Physiol., **1**, Abt. 1.

[4] Hofmeister's Beiträge, **4**.

[5] Zeitschr. f. physiol. Chem., **34**.

[6] Mörner, Centralbl. f. Physiol., **7**; Langstein, Münch. med. Wochenschr., 1902, 1876, and Wien. Sitzungsber., **112**, Abt. II*b*, 1903; Monatsheft f. Chem., **25**; Hofmeister's Beiträge, **6**; see also footnote 5, p. 84.

and carbohydrate acids of unknown kinds. It has not been shown whether these small amounts of carbohydrate are derived from the globulin or from other contaminating bodies. According to ZANETTI and also BYWATERS, the blood-serum contains a glucoproteid, *seromucoid*, and the investigations of EICHHOLZ [1] seem to show that the globulins are contaminated by a glucoproteid. According to LANGSTEIN the sugar is not only mixed with the globulin, but it exists in a combined form, probably in loose combination.

Serglobulin (the euglobulin) may be easily separated as a fine flocculent precipitate from blood-serum by neutralizing or making faintly acid with acetic acid and then diluting with 10–20 vols of water. For further purification this precipitate is dissolved in dilute common salt solution, or in water with the aid of the smallest possible amount of alkali, and then reprecipitated by diluting with water or by the addition of a little acetic acid. All the serglobulin may also be separated from the serum by means of magnesium or ammonium sulphate; in these cases it is difficult to completely remove the salt by dialysis. As long as we are not agreed as to the number of globulins in the serum, it is not necessary to give a method of separating the various globulins in this mixture. Thus far the fractional precipitation with $(NH_4)_2SO_4$ has chiefly been used. The serglobulin from blood-serum is always contaminated with lecithin and thrombin. A serglobulin free from thrombin may be prepared from ferment-free transudates, as sometimes from hydrocele fluids, and this shows that serglobulin and thrombin are different bodies. For the detection and the quantitative estimation of serglobulin we may use the precipitation by magnesium sulphate added to saturation (HAMMARSTEN), or by an equal volume of a saturated *neutral* ammonium-sulphate solution (HOFMEISTER and KAUDER and POHL [2]). In the quantitative estimation the precipitate is collected on a weighed filter, washed with the salt solution employed, dried with the filter at about 115° C., then washed with boiling-hot water, so as to completely remove the salt, extracted with alcohol and ether, dried, weighed, and incinerated to determine the ash. This method, according to the investigations of WIENER,[3] can only be used when the serum is sufficiently diluted with water.

Seralbumins are found in large quantities in blood-serum, blood-plasma, lymph, transudates, and exudates. Probably they also occur in other animal fluids and tissues. The proteins which pass into the urine under pathological conditions consist largely of seralbumin.

The seralbumin, like the serglobulin, seems also to be a mixture of at least two protein bodies. The preparation of crystalline seralbumin

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[1] Zanetti, Chem. Centralbl., 1898, I, p. 624; Bywaters, Journ. of Physiol., **35**, and Biochem. Zeitschr., **15**; Eichholz, Journ. of Physiol., **23**.

[2] Hammarsten, l. c.; Hofmeister, Kauder and Pohl, Arch. f. exp. Path. u. Pharm., **20**.

[3] Zeitschr. f. physiol. Chem., **74**.

(from horse-serum) was first performed by GÜRBER. It crystallizes with difficulty from other blood-sera (GRUŽEWSKA). Even from horse-serum only a portion, according to ROBERTSON [1] not more than 40 per cent, of the albumin can be obtained as crystals, and it is also possible that the amorphous albumin, which is precipitated by ammonium sulphate with difficulty, represents two seralbumins (MAXIMOWITSCH). According to GÜRBER and MICHEL it would seem that the crystalline seralbumin is also a mixture, but this is disproved by the obsertions of SCHULZ, WICHMANN, and KRIEGER [2]. We know nothing as to the behavior of the amorphous fraction of the seralbumin in this respect. Because of the different coagulation temperatures, HALLIBURTON claims the existence of three different albumins in the blood-serum, a view which has been disputed by several experimenters, and recently by HOUGARDY. On the other hand, the earlier investigations of KAUDER, as well as the more recent work of OPPENHEIMER,[3] seem to indicate a non-unit nature of the seralbumins, but this question is still an open one.

The crystalline seralbumin may perhaps be a combination with sulphuric acid (K. MÖRNER, INAGAKI). The coagulated albumin obtained from the aqueous solution of the crystals with the aid of alcohol has almost the same elementary composition (MICHEL) as the amorphous mixture of albumin prepared from horse-serum (HAMMARSTEN and K. STARKE [4]). The average composition was C 53.06, H 6.98. N 15.99, S 1.84 per cent. K. MÖRNER, after the removal of the sulphuric acid from crystalline albumin, found 1.73 per cent total sulphur, which probably exists only as cystine. LANGSTEIN [5] has been able to split off a nitrogenous carbohydrate (glucosamine) from crystalline seralbumin. The quantity was so small that the question is still undecided whether or not the carbohydrate was a contamination. The fact that ABDERHALDEN, BERGELL, and DÖRPINGHAUS [6] were able to prepare a seralbumin entirely free from carbohydrate and which did not respond to MOLISCH's very delicate reaction, seems to be decisive on this point. The specific rotation of crystalline seralbumins from horse-serum was found by MICHEL to be $(\alpha)_D = -61$ to 61.2°, and by MAXIMOWITSCH on the contrary $(\alpha)_D = -47.47°$.

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[1] Journ. of biol. Chem., **13**.

[2] In regard to the literature on the crystalline seralbumins, see Schulz, Die Kristallisation von Eiweissstoffen, Jena, 1901; Maximowitsch, Maly's Jahresber., **31**, 35.

[3] Halliburton, Journ. of Physiol., **5** and **7**; Hougardy, Centralbl. f. Physiol., **15**, 665; Oppenheimer, Verhandl. d. physiol. Gesellsch., Berlin, 1902.

[4] Michel, Verhandl. d. phys-med. Gesellsch. zu Würzburg, **29**, No. 3; K. Starke, Maly's Jahresber., **11**; K. Mörner, l. c.; Inagaki, Biochem, Centralbl., **4**, p. 515.

[5] K. Mörner, l. c.; Langstein, Hofmeister's Beiträge, **1**.

[6] Zeitschr. f. physiol. Chem., **41**.

The crystalline and amorphous seralbumin in aqueous solution give the ordinary albumin reactions. The coagulation temperature of a 1-per cent solution, poor in salts is about 50° C., but rises with the quantity of salt. The coagulation of the mixture of albumins from serum generally takes place at 70–85° C., but is essentially dependent upon the reaction and the amount of salt present. Up to the present time no seralbumin solution has been prepared free from mineral bodies. A solution as free from salts as possible does not coagulate either on boiling or on the addition of alcohol. On the addition of a little common salt it coagulates in both cases.[1]

Seralbumin differs from the albumin of the white of the hen's egg in the following particulars: It is more levogyrate; the precipitate formed by hydrochloric acid easily dissolves in an excess of the acid; it is rendered less insoluble by alcohol.

In preparing the seralbumin mixture, first remove the globulins, according to JOHANSSON, by saturating with magnesium sulphate at about 30° C. and filtering at the same temperature. The cooled filtrate is separated from the crystallized salt and is treated with acetic acid so that it containes about 1 per cent. The precipitate formed is filtered off, pressed, dissolved in water with the addition of alkali to neutral reaction, and the solution freed from salt by dialysis. The mixture of albumins may be obtained in a solid form from the dialyzed solution, either by evaporating the solution at a gentle temperature or by precipitating with alcohol, which must be quickly removed. STARKE[2] has suggested another method, which is also to be recommended. The crystalline seralbumin may be prepared from serum freed from globulin by half saturating with ammonium sulphate, by the addition of more salt until a cloudiness appears, and then proceeding according to the suggestion of GÜRBER and MICHEL. On acidifying with acetic acid or sulphuric acid the crystallization may be considerably accelerated.[3] The quantity of seralbumin is best calculated as the difference between the total proteins and the globulins. A method for the quantitative estimation of globulins and albumins in blood serum by refractometric means has been suggested by ROBERTSON.[4]

Summary of the elementary composition of the above-mentioned and described proteins (from horse-blood):

	C	H	N	S	O	
Fibrinogen	52.93	6.90	16.66	1.25	22.26	(HAMMARSTEN)
Fibrin	52.68	6.83	16.91	1.10	22.48	"
Fibrin-globulin	52.70	6.98	16.06	....	.....	"
Serglobulin	52.71	7.01	15.85	1.11	23.32	"
Seralbumin	53.08	7.10	15.93	1.90	21.86	(MICHEL)

[1] In regard to the relationship of neutral salts to heat coagulation,, see J. Starke, Sitzungsber. d. Gesellsch. f. Morph. u. Physiol. in München. 1897.

[2] Johansson, Zeitschr. f. physiol. Chem., 9; K. Starke, Maly's Jahresber., 11.

[3] See Hopkins and Pinkus, Journ. of Physiol., 23; Krieger, Ueber die Darstellung krystallinscher tierischer Eiweissstoffe, Inaug.-Dissert. Strassburg, 1899.

[4] Journ. of biol. Chem., 11.

Proteose-like substances have been found in blood-serum by several investigators, and NOLF [1] has shown that after the abundant introduction of proteoses into the intestine, they pass into the blood. BORCHARDT [2] has also been able to show that not only after the introduction of elastin-proteose *per os*, but also after feeding dogs with not over-abundant quantities of elastin, a proteose, hemielastin, passes into the blood and can indeed be eliminated in the urine. The question whether the proteoses are normal constituents of the blood under ordinary conditions is still much disputed. The difficulty in deciding this question lies in the fact that in the removal of the proteins a small amount of proteose-like substance is formed from other proteins (namely from the globin of the blood pigment), and on the other hand the proteoses can be precipitated with the other bodies. The question as to the physiological occurrence of proteoses in the blood or plasma must be considered as still undecided.[3]

In close relation to the proteoses stands perhaps the above-mentioned seromucoid, which was discovered by ZANETTI and especially studied by BYWATERS. It is a glycoprotein which is soluble in water, and precipitated by alcohol. Seromucoid contains, according to BYWATERS,[4] 11.6 per cent N, 1.8 per cent S, and yields approximately 25 per cent glucosamine. The quantity in the blood is 0.2–0.9 p. m.

The Blood-serum.

As above stated, the blood-serum is the clear liquid which is pressed out by the contraction of the blood-clot. It differs chiefly from the plasma in the absence of fibrinogen and in containing an abundance of fibrin ferment. Otherwise considered qualitatively, the blood-serum contains the same chief constituents as the blood-plasma.

Blood-serum is a sticky liquid which is more alkaline toward litmus than the plasma. The specific gravity in man is 1.027 to 1.032, average 1.028. The color is more or less yellow; in human blood-serum it is pale yellow with a shade toward green, and in horses it is often amber-yellow. The serum is ordinarily clear; after a meal it may be opalescent, cloudy, or milky white, according to the amount of fat contained in the food.

Besides the above-mentioned bodies, the following constituents are found in the blood-plasma or blood-serum:

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[1] Bull. Acad. Roy. Belg., 1903 and 1904.

[2] Zeitschr. f. physiol. Chem., 51 and 57.

[3] See especially Abderhalden, Zeitschr. f. physiol. Chem., 51, and Biochem. Zeitschr., 8 and 10, and E. Freund, *ibid.*, 7 and 9, which also contains the literature.

[4] Biochem. Zeitschr., 15.

Fat occurs from 1–7 p. m. in fasting animals. After partaking of food the amount is increased to a great extent. *Fatty acids*, or *soaps*, *glycerin* (NICLOUX, FR. TANGL, and ST. WEISER [1]) *phosphatides* and *cholesterin* are also found. Cholesterin occurs, according to HÜRTHLE [2], at least in part, as fatty-acid esters (*serolin* according to BOUDET). According to LETSCHE [3] free cholesterin probably also occurs in the serum.

Sugar seems to be a physiological constituent of the plasma and serum. According to the investigations of many workers [4] the sugar found is glucose. STRAUSS [5] has also detected fructose in blood-serum and in transudates and exudates. The question as to the occurrence of other varieties of sugar, such as isomaltose (PAVY and SIAU) and pentose (LÉPINE and BOULUD [6]), in blood serum is still undecided. ASHER and ROSENFELD and MICHAELIS and RONA in a more conclusive manner, have shown that at least a considerable part of the sugar can be removed from the blood by dialysis, hence it must exist in solution in the free state. These observations do not exclude the possibility of the existence of another part of the sugar which is in combination with protein. LÉPINE and BOULUD [7] could only obtain a diffusion of the sugar by a short dialysis from serum 12 hours old, but not from perfectly fresh serum, an observation which somewhat diminishes the conclusiveness of MICHAELIS and RONA's experiment with 24-hour dialysis. A further testing of this question is therefore very desirable.

The quantity of sugar in the serum or plasma is for man 0.6–1 p. m. calculating the total reduction as glucose, and in animals about the same but in rabbits considerably higher or 2.2 p. m.[8] Besides the sugar, the blood contains, as first shown by J. OTTO, also another or perhaps several reducing substances, a part existing in the serum and another part in the blood-corpuscles. We will discuss the nature of these bodies as well as the so-called virtual sugar and glycolysis in speaking of the division of the sugar in the blood-corpuscles and plasma in connection with

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[1] Nicloux, Compt. Rend. soc. biol., **55**; Tangl and St. Weiser, Pflüger's Arch., **115**.

[2] Hürthle, Zeitschr. f. physiol. Chem., **21**, where Boudet is also cited. In regard to the quantity of these esters in bird-serum, see Brown, Amer. Journ. of Physiol., **2**.

[3] Zeitschr. f. physiol. Chem., **53**.

[4] See v. Mering, Arch. f. (Anat. u.) Physiol., 1877 (this article contains numerous references); Seegen, Pflüger's Arch., **40**; Miura, Zeitschr. f. Biologie, **32**.

[5] Fortschritte d. Medic., 1902.

[6] Pavy and Siau, Journ. of Physiol., **26**; Lepine and Boulud, Compt. Rend., **132**, **135**, and **136**.

[7] Rosenfeld, Centralbl. f. Physiol., **19**, p. 449; Lépine and Boulud, Compt. Rend., **142**; Asher, Biochem. Zeitschr., **3**; Michaelis and Rona, *ibid.*, 14.

[8] See E. Frank, Zeitschr. f. physiol. Chem., **70**; Lyttkens and Sandgren, Bioch. Zeitschr., **21** and **26**.

the total blood. The same applies to the conjugated glucuronic acids, which it seems, originate from the form-elements.

The blood-plasma and the serum, as well as the lymph also contain *enzymes* of various kinds. According to RÖHMANN, BIAL, HAMBURGER,[1] and others, *diastases*, which convert starch and glycogen into maltose or isomaltose, as well as a *maltase*, are found in the blood. The diastase, whose quantity is very variable in the blood of different animals, seems at least in great part, to originate in the pancreas but can also come from other organs and according to HABERLANDT also from the leucocytes.[2] HANRIOT and others have detected, in the serum, *lipases* or *esterases* which decompose butyrin and neutral fats and other esters. The occurrence of *butyrinases* which split mono- as well as tributyrin has been recently substantiated by RONA and MICHAELIS, while the property of this lipase of splitting olein and other neutral fats is not generally acknowledged (ARTHUS, DOYON and MOREL[3]).

This lipolytic property, if it exists to the extent that HANRIOT ascribes to it, must not be confounded with the transformation of fat into unknown substances soluble in water, a phenomenon first observed by CONNSTEIN and MICHAELIS and further studied by WEIGERT. The occurrence of such a body is positively denied by G. MANSFELD.[4]

Besides the above-mentioned enzymes and thrombin and the glycolytic enzymes that will be discussed later, several other enzymes have been found in the blood-serum, namely, *oxidases*, *catalases*, *proteolytic enzymes*, among which we must mention the *polypeptide-splitting enzymes* studied by ABDERHALDEN and collaborators,[5] also *rennin* and several *antienzymes*. We cannot enter into the discussion of these, nor of the many not chemically characterized bodies which have been called *toxines* and *antitoxines*, *immune bodies*, *alexines*, *hæmolysines*, *cytotoxines*, etc., and which have been discussed in Chapter I. The various enzymes and antienzymes, and the above mentioned bodies are as a rule precipitated with the globulin, but differ among each other in that some are

[1] Röhmann; Röhmann and Hamburger, Ber. d. deutsch. chem. Gesellsch., **25** and **27**; Pflüger's Arch., **52** and **60**; Bial, Ueber das diast. Ferm., etc., Inaug.-Diss. Breslau, 1892 (older literature). See also Pflüger's Arch., **52**, **54**, and **55**; Wohlgemuth, Bioch. Zeitschr., **21**.

[2] Wohlgemuth, l. c.; Moeckel and Rost, Zeitschr. f. physiol. Chem., **67**; Clerk and Loeper, Compt. Rend. soc. biol., **66**; Haberlandt, Pflüger's Arch., **132**.

[3] Hanriot, Compt. Rend. soc. biol., 48 and 54, Compt. Rend. **123** and **132**; Rona, and Michaelis, Bioch. Zeitschr., **31**; Rona, *ibid.*, **33**; Arthus, Journ. de Physiol. et de Pathol., **4**; Doyon and Morel, Compt. Rend. soc. biol., **54**; Achard and Clerç (Lipase in Disease), Compt. Rend., **129**, and Arch. d. med. expér., **14**.

[4] Connstein and Michaelis, Pflüger's Arch., **65** and **69**; Weigert, *ibid.*, **82**; Mansfeld, Centralbl. f. Physiol., **21**.

[5] Zeitschr. f. physiol. Chem., **51**, **53**, **55**.

carried down by the euglobulin, while the others are carried down by the pseudoglobulin fraction.

The non-protein organic constituents of the serum have been given especial and careful study by E. LETSCHE [1] and he has found, besides the previously known bodies, that the serum contains several acids, among which there are two nitrogeneous acids whose nature has not been studied. These, including other nitrogenous substances found by him, represent a part of the so-called *rest nitrogen*, i.e., that nitrogen which remains in the serum after the complete removal of the coagulable proteins. As representatives of the bodies occurring as rest nitrogen in the serum we must in the first place mention *urea*, also *creatine*, *carbamic acid*, *ammonia*, *hippuric acid*, *phosphocarnic acid* (PANELLA), traces of *indol* (HERVIEUX), perhaps also *uric acid* found by ABELES [2] in human blood, while LETSCHE could not find any in horse-blood.

According to BROWINSKI proteic acids (see Chapter XIV) occur in the serum and CZERNECKI [3] has investigated the quantity of proteic acid nitrogen in serum and transudates under different conditions. The occurrence of proteoses is, as above mentioned, somewhat disputed. We have several investigations on the occurrence of amino-acids (v. BERGMANN, HOWELL, LETSCHE, ABDERHALDEN and others) which make the occurrence of these very probable, and recently BINGEL has been able to show the presence of glycocoll in normal ox-blood. Otherwise the amino-acids have often been sought for in normal blood but in vain; still recently certain investigators like VAN SLYKE and MEYER [4] have shown the presence of amino-acids in the blood under normal conditions. In dog blood after 24 hours' starvation they found 3–5 milligrams of amino-acid nitrogen in 100 parts blood. Under pathological conditions *lysine* (NEUBERG and RICHTER [5]), *leucine* and *tyrosine* have been found. Also *purine bases* and *bile acids* have been found in the serum under pathological conditions. That the quantity of rest nitrogen is larger during digestion than in starvation requires further confirmation.[6]

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[1] Zeitschr. f. physiol. Chem., **53**.

[2] Panella, cited in Virchow's Jahresb., 1902, 150, Hervieux Compt. Rend. soc. biol., **56**; Abeles, Wien. med. Jahrb., 1887.

[3] Browinski, Zeitschr. f. physiol. Chem., **54** and **58**; Czernecki, Maly's Jahresb., **39** and **40**.

[4] v. Bergmann, Hofmeister's Beiträge, **6**; Howell, Amer. Journ. of Physiol., **17**; Letsche, l. c.; Abderhalden, Zeitschr. f. physiol. Chem., **72**; Bingel, *ibid.*, **57**; D. v. Slyke and Meyer, Journ. of biol. Chem., **12**.

[5] Deutsch. med. Wochenschr., 1904.

[6] v. Bergmann and Langstein, Hofmeister's Beiträge, **6**; Hohlweg and Meyer, *ibid.*, **11**.

As rest-carbon MANCINI [1] designates that carbon which is not precipitated by phosphotungstic acid. It originates in great part from the urea and sugar and amounts to 0.076–0.089 gram in 100 cc.

The *pigments* of the blood-serum are very little known. Besides other pigments horse-serum contains, as first shown by HAMMARSTEN, bilirubin, which, according to RANC, is the only pigment of the serum of this animal. This pigment occurs, although in small amounts, sometimes in the serum of other animals and, according to BIFFI and GALLI,[2] is especially abundant in the blood of new-born.

Urobilin is not, according to AUCHÉ, ROTH and HERZFELD, a physiological serum-pigment. Urobilinogen may occur in extraordinary cases according to HILDEBRANDT,[3] and on allowing the blood in such cases to stand urobilin may be formed therefrom. The yellow coloring-matter of the serum seems to belong to the group of *luteins*, which are often called *lipochromes* or fat-coloring matters. From ox-serum KRUKENBERG [4] was able to isolate with amyl alcohol a so-called lipochrome whose solution shows two absorption-bands, of which one encloses the line *F* and the other lies between *F* and *G*.

The *mineral bodies* in serum and plasma are qualitatively, but not quantitatively, the same. A part of the calcium, magnesium, and phosphoric acid is removed on the coagulation of the fibrin. By means of dialysis, the presence of sodium chloride, which forms the chief mass or 60–70 per cent of the total mineral bodies, lime-salts, sodium carbonate, and traces of sulphuric and phosphoric acids and of potassium, may be directly shown in the serum.[5] Traces of silicic acid, fluorine, copper, iron, and manganese, are claimed to have been found in the serum. As in most animal fluids, the chlorine and sodium are in the blood-serum in excess of the phosphoric acid and potassium (the occurrence of which in the serum is even doubted). The acids present in the ash are not sufficient to saturate the bases found, a condition which shows that a part of the bases is combined with organic substances, perhaps proteins. This also coincides with the fact that the great part of the alkalies does not exist in the serum as diffusible alkali compounds, carbonate and phosphate, but as non-diffusible compounds, protein combinations. According to HAMBURGER 37 per cent of the alkali of the serum from horse-blood was diffusible and 63 per cent non-diffusible.

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[1] Bioch. Zeitschr., **26** and **32**.

[2] Hammarsten, see Maly's Jahresb., 8 (1878); Ranc, Compt. Rend. soc. biol., **62**; Biffi and Galli, Journ. de Physiol. et Path., **9** (1907).

[3] Auché, Compt. Rend. soc. biol., **67**; Roth and Herzfeld, Deutsch. med. Wochenschr., **37**; Hildebrandt, Münch. Med. Wochenschr., **57**.

[4] Sitz.-Ber. d. Jen. Gesellsch. f. Med., 1885.

[5] See Gürber, Verhandl. d. phys.-med. Gesellsch. zu Wurzburg, **28**.

According to RONA and TAKAHASHI [1] 25–30 per cent of the calcium is non-diffusible, probably combined with proteins.

Iodine, which seems to be habitually found, is also considered as a mineral constituent of the plasma or serum (GLEY and BOURCET), while arsenic, although not found in all blood, occurs in human blood (GAUTIER, BOURCET [2]). Iodine occurs to a greater extent in menstrual blood than in other blood and does not exist as a salt, but as an organic compound (BOURCET).

The *gases* of the blood-serum, which consists chiefly of carbon dioxide with only a little nitrogen and oxygen, will be described when treating of the gases of the blood.

We have only a few analyses of blood-plasma. As an example the results of the analyses of the blood-plasma of the horse will be given below. The analysis No. 1 was made by HOPPE-SEYLER.[3] No. 2 is the average of the results of three analyses made by HAMMARSTEN. The figures are given for 1000 parts of the plasma.

	No. 1.	No. 2.
Water	908.4	917.6
Solids	91.6	82.4
Total proteins	77.6	69.5
Fibrin	10.1	6.5
Globulin	.....	38.4
Seralbumin	.....	24.6
Fat	1.2	12.9
Extractive substances	4.0	
Soluble salts	6.4	
Insoluble salts	1.7	

LEWINSKY [4] has determined the total proteins and the individual proteins in the blood-plasma of man and animals with the following results:

	Total Protein.	Albumin.	Globulin.	Fibrinogen.
Man	72.6	40.1	28.3	4.2
Dog	60.3	31.7	22.6	6.0
Sheep	72.9	38.3	30.0	4.6
Horse	80.4	28.0	47.9	4.5
Pig	80.5	44.2	29.8	6.5

ABDERHALDEN has made complete analyses of the blood-serum of several domestic animals. From these analyses, as well as from those made by HAMMARSTEN of the serum from human, horse, and ox-blood, it follows that the amount of solids ordinarily varies between 70–97 p. m. The chief mass of the solids consists of proteins, about 55–84 p. m. In hens HAMMARSTEN found much lower values, namely, 54

[1] Hamburger, Arch. f. (Anat. u.) Physiol., 1898; Rona and Takahashi, Bioch. Zeitschr., 31.

[2] Gley and Bourcet, Compt. Rend., 130; Bourcet, *ibid.*, 131; Gautier, *ibid.*, 131.

[3] Cit. from v. Gorup-Besanez's Lehrbuch der physiol. Chem., 4. Aufl., 346.

[4] Pflüger's Arch., 100.

p. m. solids, with only 39.5 p. m. protein, and HALLIBURTON found only 25.4 p. m. protein in frog's blood. The relation between globulin and seralbumin is, as shown by the analyses of HAMMARSTEN, HALLIBURTON, and RUBBRECHT,[1] very different for various animals, but may also vary considerably in the same species of animal. In human blood-serum HAMMARSTEN found more seralbumin than globulin, and the relation of serglobulin to seralbumin was as 1:1.5. LEWINSKY found the relationship in man greater than 1, indeed 1:1.39–2.13. In regard to the quantity of the remaining organic constituents of the serum we refer the reader to ABDERHALDEN'S complete analyses.

In starvation it seems, as first found by BURCKHARDT and then substantiated by other investigators, that the quantity of globulins relative to that of albumin in dogs and also in rats (ROBERTSON[2]), is increased. According to ROBERTSON, in the horse, ox and rabbit the reverse exists, namely, the amount of albumin relative to the globulin increases in starvation. A change in the relation with a decrease in the albumin and an increase in the globulin may also occur in animals which have been made sick or in part immune by inoculation with pathogenic micro-organisms (LANGSTEIN and MAYER[3]). The total protein content is raised in nearly all cases. The amount of fibrinogen in the plasma is especially increased by pneumococci, streptococci, and pus-staphylococci (P. MÜLLER[4]).

The quantity of mineral bodies in the serum has been determined by many investigators. The conclusion drawn from the analyses is that there exists a rather close correspondence between human and animal blood-serum, and it is therefore sufficient to here give the analysis of C. SCHMIDT[5] of (1) human blood, and BUNGE and ABDERHALDEN'S analyses (2) of serum of ox, bull, sheep, goat, pig, rabbit, dog, and cat. The results correspond to 1000 parts by weight of the serum.

	1	2
K_2O	0.387–0.401	0.226–0.270
Na_2O	4.290–4.290	4.251–4.442
Cl	3.565–3.659	3.627–4.170
CaO	0.155–0.155	0.119–0.131
MgO	0.101	0.040–0.046
P_2O_5 (inorg.)	.....	0.052–0.085

[1] Abderhalden, Zeitschr. f. physiol. Chem., **25**; Hammarsten, Pflüger's Arch., **17**; Halliburton, Journ. of Physiol., **7**; Rubbrecht, Travaux du laboratoire de l'institut de physiologie de Liege, **5**, 1896.

[2] Burckhardt, Arch. f. exp. Path. u. Pharm., **16**; Githens, Hofmeister's Beiträge, **5**; see also Morawitz, *ibid.*, **7**, and Inagaki, Zeitschr. f. Biol., **49**; Robertson, Journ. of biol. Chem., **13**.

[3] Hofmeister's Beiträge, **5**.

[4] *Ibid.*, **6**.

[5] Cit. from Hoppe-Seyler, Physiol. Chem., 1881, p. 439.

A MACALLUM[1] has determined the quantity of mineral bodies in the serum of certain cold-blooded animals (fishes, shark, lobster and others). The amount of sodium and chlorine in the serum of these animals living in sea-water was much greater than in warm-blooded animals.

Even if we bear in mind that certain bodies, such as carbon dioxide, are driven off during incineration, and that other bodies, such as sulphuric acid and phosphoric acid, are formed from sulphurized and phosphorized organic substances, still quantitative analyses like the above are not sufficient for the scientific demands of to-day. They do not show the true composition, and especially do not give an explanation of the number of different ions present in the serum or in other fluids, a question which is of the greatest physiological importance. An answer to these questions is obtainable only by physico-chemical investigations, which have thus far been used chiefly in determining the molecular concentration, the amount of electrolytes and non-electrolytes, and the degree of dissociation.

The average depression of the freezing-point of mammalian blood corresponds, as given in Chapter I, closely to a 9 p. m. (Δ = about $-0.56°$) solution of common salt, and at the present time such a solution is considered as a physiological salt solution for man and other mammalia. In lower animals and fish the conditions are otherwise, as shown in the above-mentioned chapter.

There are recorded a great number of investigations on the changes in the osmotic pressure or the molecular concentration of the blood-serum under various physiological conditions as well as in disease, but still it is no doubt too early to draw any definite conclusions from these observations.

The degree of dissociation (see Chapter I) of sera has been determined by several investigators, and according to HAMBURGER[2] it lies between 0.65 and 0.82. The molecular concentration, which represents the total number of molecules and ions per liter, is according to BURGARSKY and TANGL, on an average about 0.320 mol. per liter. They also found that about three-fourths of the total number of dissolved molecules in blood-serum were electrolytes, although the serum contained about 70–80 p. m. protein and 10 p. m. inorganic bodies, and also that three-fourths of the quantity of electrolytes consisted of NaCl.

In the determination of the alkalinity of blood and blood-serum, up to the present time, we have estimated the amount of alkali by titration with an acid. We cannot dispense with such determinations, although

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[1] Proc. Roy. Soc., ser. B., 82.

[2] Osmotischer Druck und Ionenlehre, Wiesbaden, 1902–1904, where the literature on the physical chemistry of the blood can be found.

they do not yield any information as to the true alkalinity, apart from the fact that the results are dependent upon the indicator used, because we understand as true alkalinity the concentration of the hydroxyl ions. The Na_2CO_3 is in aqueous solution more or less dissociated into $2Na^+$ and $CO_3^=$, depending upon the dilution. The $CO_3^=$ ions combine partly with the H^+ ions of the dissociated water, forming HCO_3^-, and the corresponding HO^- ions produce the alkaline reaction. If now by the addition of a little acid, a few of the HO^- ions are removed, the equilibrium is then disturbed, a new quantity of Na_2CO_3 is dissociated, and this process is repeated every time a new quantity of acid is added until all the carbonate is dissociated. The dissociation of the carbonate existing in the original concentration, upon which the number of HO^- ions is dependent, cannot therefore be determined by titration.

For these reasons we generally determine the quantity of HO and H ions in the serum and blood by methods based upon NERNST'S theory for the electromotive force of gas-chains. According to these investigations it has been found that the concentration of the hydroxyl ions in blood-serum and blood is only a little higher than in distilled water (see Chapter I page 76, and the reaction of the blood below).

II. THE FORM-ELEMENTS OF THE BLOOD.

The Red Blood-corpuscles.

The blood-corpuscles are round, biconcave disks without membrane and nucleus, in man and mammalia (with the exception of the llama, the camel, and their congeners). In the latter animals, as also in birds, amphibia, and fish (with the exception of the Cyclostoma) the corpuscles have in general a nucleus, are biconvex and more or less elliptical. The size varies in different animals. In man they have an average diameter of 7 to 8 μ (μ=0.001 mm.) and a maximum thickness of 1.9 μ. They are heavier than the blood-plasma or serum, and therefore sink in these liquids. In the discharged blood they may sometimes lie with their flat surfaces together, forming a cylinder like a roll of coin (rouleaux). The reason for this phenomenon, which is considered as an agglutination, has not been sufficiently studied, but as it may be observed in defibrinated blood it seems probable that the formation of fibrin has nothing to do with it.

The number of red blood-corpuscles is different in the blood of various animals. In the blood of man there are generally 5 million red corpuscles in 1 c.mm., and in woman 4 to 4.5 million.

The blood-corpuscles consist principally of two chief constituents, the stroma, which forms the real protoplasm, and the intraglobular contents, whose chief constituent is hæmoglobin. We cannot state

anything positive for the present in regard to a more detailed arrangement, and the views on this subject are somewhat divergent. The two following views are more or less related to each other. According to one view the blood-corpuscles consist of a membrane which encloses a hæmoglobin solution, while the other view considers the stroma as a protoplasmic structure soaked with hæmoglobin. This latter view is in accord with the assumption as to an outside boundary-layer. Thus according to HAMBURGER the stroma forms a protoplasmic net in whose meshes there exists a red fluid or semi-fluid mass which consists in great measure of hæmoglobin. This mass represents the water-attracting force of the blood-corpuscles, and besides this it is also considered that the outer protoplasmic boundary is semi-permeable, i. e., permeable to water but not permeable to certain crystalloids. The researches of KÖPPE, ALBRECHT, PASCUCCI, RYWOSCH,[1] and others indicate the presence of a special envelope or boundary-layer, and there is no doubt that the outer layer contains so-called lipoids, such as cholesterin, lecithin, and similar bodies.

The red blood-corpuscles retain their volume in a salt solution which has the same osmotic pressure as the serum of the same blood, although they may change their form in such solutions, becoming more spherical, and may also undergo a chemical change. Such a salt solution is *isotonic* with the blood-serum, and its concentration for a NaCl solution is approximately 9 p. m. for human and mammalian blood. A solution of greater concentration, a *hyperisotonic* solution, abstracts water from the blood-corpuscles until osmotic equilibrium is established, hence the corpuscles shrink and their volumes become smaller. In solutions of less concentration, *hypisotonic* solutions, the corpuscles swell, due to the taking up of water, and this swelling may be so great, on diluting the blood with water, that the hæmoglobin is separated from the stroma and passes into the watery solution. This process is called *hæmolysis* (see Chapter I).

A hæmolysis may also be brought about by alternately freezing and thawing the blood, as well as by the action of various chemical substances, which act as protoplasmic poisons. These bodies are ether, chloroform alkalies, bile-acids, solanin, saponin, and also the saponin substances, which have a very strong hæmoloytic action, also metabolic products of bacteria, higher plants and animals (snakes, toads, bees, spiders and others) and also bodies occurring in blood serum of normal or immunized animals.

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[1] See Hamburger, Osmotischer Druck und Ionenlehre, 1902; Köppe, Pflüger's Arch., **99** and **107**; Albrecht, Centralbl. f. Physiol., **19**; Pascucci, Hofmeister's Beiträge, **6**; Rywosch, Centralbl. f. Physiol., **19**.

When the hæmoglobin is separated from the so-called stroma by a sufficiently strong dilution with water the stroma is found in the solution in a swollen condition. By the action of carbon dioxide, by the careful addition of acids, acid salts, tincture of iodine, or certain other bodies, this residue, rich in proteins, condenses, and in many cases the form of the blood-corpuscles may be again obtained. This residue, the so-called ghosts or stromata of the blood-corpuscles, can also be directly colored in dilute blood by methyl violet and in this way detected, and attempts have been made to isolate it for chemical investigation. In the following pages we mean by the name stroma only that residue which remains after the removal of hæmoglobin and other bodies soluble in water.

To isolate the stromata from the blood-corpuscles, they are washed first by diluting the blood with 10–20 vols. of a 1–2 per cent common salt solution and then separating the mixture by centrifugal force or by allowing it to stand at a low temperature. This is repeated a few times until the blood-corpuscles are freed from serum. These purified blood-corpuscles are, according to WOOLDRIDGE, mixed with 5–6 vols. of water and then a little ether is added until complete solution is obtained. The leucocytes gradually settle to the bottom, a movement which may be accelerated by centrifugal force, and the liquid which separates therefrom is very carefully treated with a 1-per cent solution of $KHSO_4$ until it is about as dense as the original blood. The separated stromata are collected on a filter and quickly washed. PASCUCCI,[1] on the contrary, treats the mass of corpuscles with 15–20 vols. of a one-fifth saturated ammonium-sulphate solution, allows the corpuscles to settle, siphons off the fluid, repeatedly centrifuges, allows the residue to dry quickly (on porcelain plates) at the ordinary temperature, and then washes with water until the blood-pigments and the other soluble bodies are dissolved out.

WOOLDRIDGE found as constituents of the stromata *lecithin*, *cholesterin*, *nucleoalbumin*, and a *globulin* which, according to HALLIBURTON, is probably a nucleoproteid which he calls *cell-globulin*. No nuclein substances or seralbumin or proteoses could be detected by HALLIBURTON and FRIEND. According to PASCUCCI, the stromata (from horse-blood) consists of one-third cholesterin and lecithin (besides a little cerebroside), and two-thirds protein substances and mineral bodies. The nucleated red blood-corpuscles of the bird contain, according to PLÓSZ and HOPPE-SEYLER,[2] a protein (nucleoprotein) which swells to a slimy mass in a 10-per cent common salt solution, and which seems to

[1] Hofmeister's Beiträge, 6.

[2] Wooldridge, Arch. f. (Anat. u.) Physiol., 1881, 387; Halliburton and Friend, Journal of Physiol., 10; Halliburton, *ibid.*, 18; Plósz, Hoppe-Seyler's Med. chem. Untersuch., 510.

be closely related to the hyaline substance (*hyaline substance* of ROVIDA), occurring in the lymph-cells. In the mass extracted by alcohol from the blood-corpuscles of the hen, ACKERMANN found 3.93 per cent phosphorus and 17.2 per cent nitrogen, which on calculation gave 42.10 per cent nucleic acid and 57.82 per cent histone. PIETTRE and VILA [1] found, in the stromata, 0.3 per cent phosphorus in the horse and 2.3–2.6 per cent in birds (ducks and hens), calculated on the ash-free substance. They found the quantity of nitrogen to be 11.7 and 13.21 per cent for the horse and dog respectively. The non-nucleated red blood-corpuscles are, as a rule, very poor in protein, but rich in hæmoglobin; the nucleated corpuscles are richer in protein and poorer in hæmoglobin than the non-nucleated. The reducing substances, and in certain animal sugars, probably also conjugated glucuronic acids and several enzymes, among which occurs the proteolytic enzyme studied by ABDERHALDEN and collaborators,[2] belong to the stromata. It is difficult to decide in many cases whether the enzymes found in the blood belong to the fluid or to the various kinds of form-elements.

A gelatinous, fibrin-like protein body may be obtained from the red blood-corpuscles under certain circumstances. This fibrin-like mass has been observed on freezing and then thawing the sediment of the blood-corpuscles, or on discharging the spark from a large Leyden jar through the blood, or on dissolving the blood-corpuscles of one kind of animal in the serum of another (LANDOIS, *stroma-fibrin*); i.e., in the so-called *hæmagglutination*, a clumping of the red blood-corpuscles into clusters takes place. This agglutination can be brought about by bodies similar to the hæmolysines and also by serum constituents produced normally or by immunization. It has not been shown that a fibrin formation from the stroma takes place, nor is it probable. Fibrinogen has been detected only in the red corpuscles of frog's blood (ALEX. SCHMIDT and SEMMER [3]).

Closely related to the anatomical and chemical structure of the erythrocytes is the question, which is important for the metabolism in the blood, as to the permeability of the erythrocytes, that is, their power of taking up substances of different kinds. This question as well as the permeability of the blood-corpuscles for anions under the influence of carbon dioxide has been discussed in Chapter I, pages 7 and 8.

The *mineral bodies* of the red corpuscles will be treated in connection with their quantitative constitution.

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[1] Ackermann, Zeitschr. f. physiol. Chem., **43**; Piettre and Vila, Compt. Rend., **143**.

[2] Zeitschr. f. physiol. Chem., **51**, **53** and **55**.

[3] Landois, Centralbl. f. d. med. Wissensch., 1874, 421; Schmidt, Pflüger's Arch., **11**, 550–559.

The constituent of the blood-corpuscles existing in greatest quantity is the red pigment hæmoglobin.

Blood-pigments.

According to HOPPE-SEYLER the coloring-matter of the red blood-corpuscles is not in a free state, but combined with some other substance. The crystalline coloring-matter, the hæmoglobin or oxyhæmoglobin, which may be isolated from the blood, is considered, according to HOPPE-SEYLER, as a cleavage product of this compound, but it acts in many ways unlike the questionable compound itself. This compound is insoluble in water and uncrystallizable. It strongly decomposes hydrogen peroxide without being oxidized itself; it shows a greater resistance to certain chemical reagents (as potassium ferricyanide) than the free coloring-matter; and, lastly, it gives off its loosely combined oxygen much more easily in vacuum than the free pigment. To distinguish between the cleavage products, the hæmoglobin, and the oxyhæmoglobin, HOPPE-SEYLER calls the compound of the blood-coloring matter of the venous blood-corpuscles *phlebin*, and that of the arterial *arterin*. Other investigators, such as H. U. KOBERT and BOHR,[2] the latter calling the pigment of the blood-corpuscles *hæmochrom*, are of a similar opinion. Since the above-mentioned combinations of the blood-coloring matters with other bodies, for example (if they really do exist) with lecithin, have not been closely studied, the following statements will apply only to the free pigment, the hæmoglobin.

The color of the blood depends in part on *hæmoglobin* and in part on a molecular combination of this substance with oxygen, the *oxyhæmoglobin*. We find in blood after asphyxiation almost exclusively hæmoglobin, in arterial blood disproportionately large amounts of oxyhæmoglobin, and in venous blood a mixture of both. Blood-coloring matters are also found in striated as well as in certain smooth muscles, and lastly in solution in different invertebrates, although this pigment is not quite identical with that from higher animals. The quantity of hæmoglobin in human blood may indeed be somewhat variable under different circumstances, but amounts to about 14 per cent on an average, or 8.5 grams for each kilo of the weight of the body.

Hæmoglobin belongs to the group of compound proteins, and yields as cleavage products, besides very small amounts of volatile fatty acids and other bodies, chiefly a protein *globin*, and a coloring-matter, *hæmo-*

[2] Hoppe-Seyler, Zeitschr. f. physiol. Chem., **13**, 479; H. U. Kobert, Das Wirbeltierblut in mikro-kristallogr. Hinsicht, Stuttgart, 1901; Bohr, Centralbl. f. Physiol., **17**, p. 688.

chromogen (about 4 per cent), containing iron, which in the presence of oxygen is easily oxidized into *hæmatin*.

As first shown by SCHUNCK and MARCHLEWSKI, and especially by the work of the latter, a close relation exists between chlorophyll and the blood-pigment, because a derivative of the first, phylloporphyrin, stands very close in certain respects to a derivative of the blood-pigment hæmatoporphyrin. By the investigations of NENCKI in conjunction with MARCHLEWSKI and ZALESKI,[1] it was shown that hæmopyrrol could be prepared from the derivatives of both the leaf-pigment and the blood-pigments by reduction, and also the investigations of PILOTY and WILLSTÄTTER on chlorophyll and blood pigments have further developed the interesting biological fact that the chlorophyll and blood pigments are closely related bodies.

The hæmoglobin prepared from different kinds of blood has not exactly the same composition, which seems to indicate the presence of different hæmoglobins. The analyses by different investigators of the hæmoglobin from the same kind of blood do not always agree with one another, which probably depends upon the somewhat varying methods of preparation. The following analyses are given as examples of the constitution of different hæmoglobins:

Hæmoglobin from the	C	H	N	S	Fe	O	
Dog	53.85	7.32	16.17	0.390	0.430	21.84	(HOPPE-SEYLER)
"	54.57	7.22	16.38	0.568	0.336	20.93	(JAQUET)
Horse	54.87	6.79	17.31	0.650	0.470	19.73	(KOSSEL)
"	51.15	6.76	17.94	0.390	0.335	23.43	(ZINOFFSKY)
Ox	54.66	7.25	17.70	0.447	0.400	19.543	(HÜFNER)
Pig	54.17	7.38	16.23	0.660	0.430	21.360	(OTTO)
"	54.71	7.38	17.43	0.479	0.399	19.602	(HÜFNER)
Guinea-pig	54.12	7.36	16.78	0.580	0.480	20.680	(HOPPE-SEYLER)
Squirrel	54.09	7.39	16.09	0.400	0.590	21.440	"
Goose	54.26	7.10	16.21	0.540	0.430	20.690	"
Hen	52.47	7.19	16.45	0.857	0.335	22.500	(JAQUET)

That the repeatedly observed quantity of phosphorus in the hæmoglobin of birds (Inoko and others) is due to a contamination has been proved by ABDERHALDEN and MEDIGRECEANU. In the hæmoglobin from the horse (ZINOFFSKY), the pig, and the ox (HÜFNER), we have 1 atom of iron to 2 atoms of sulphur, while in the hæmoglobin from the dog (JAQUET) the relation is 1 to 3. From the data of the elementary analysis, as also from the amount of loosely combined oxygen, HÜFNER[1] has calculated the molecular weight of dog-hæmoglobin as 14,129, and

[1] Schunck and Marchlewski, Annal. d. Chem. u. Pharm., **278**, **284**, **288**, **290**; Nencki, Ber. d. deutsch. chem. Gesellsch., **29**; Marchlewski, and Nencki, Ber. d. d. chem., Gesellsch., **34**; Nencki and Zaleski, *ibid.*, Marchlewski, Chem. Centralbl., 1902, I, 1016; Zaleski, Zeitschr. f. physiol. Chem., **37**. The literature and the works of Willstätter and Piloty will be given under haemopyrrol, page 297.

the formula $C_{636}H_{1025}N_{164}FeS_3C_{181}$. According to the more recent determinations of HÜFNER and JAQUET, ox-hæmoglobin contains an average of 0.336 per cent iron, and the human hæmoglobin, according to BUTTERFIELD [1] contains 0.334 per cent iron. From the iron a molecular weight of 16,669 may be calculated. BARCROFT and HILL have arrived at exactly the same value by using an entirely different method and HÜFNER and GANSSER [2] have attempted to learn the size of the molecular weight of hæmoglobin by means of osmotic pressure determinations, and they found the following approximate results: for horse-hæmoglobin 15,115 and for ox-hæmoglobin 16,321. The hæmoglobin from various kinds of blood not only shows a diverse constitution, but also a different solubility and crystalline form, and a varying quantity of water of crystallization; hence we infer that there are several kinds of hæmoglobin. BOHR is a very zealous advocate of this supposition. He has been able to obtain hæmoglobins from dog- and horse-blood, by fractional crystallization, which had different powers of combining with oxygen and contained different quantities of iron. HOPPE-SEYLER had already prepared two different forms of hæmoglobin crystals from horse-blood, and BOHR concludes from all these observations that the ordinary hæmoglobin consists of a mixture of different hæmoglobins. In opposition to this statement, HÜFNER [3] has shown that only one hæmoglobin exists in ox-blood, and that this is probably true for the blood of many other animals.

Oxyhæmoglobin, which has also been called HÆMATOGLOBULIN or HÆMATOCRYSTALLIN, is a molecular combination of hæmoglobin and oxygen. For each molecule of hæmoglobin 1 molecule of oxygen is present, as shown by the investigations of HÜFNER as well as HÜFNER and GANSSER, and the amount of loosely combined oxygen which is united to 1 gram of hæmoglobin (of the ox) has been determined by HÜFNER [4] as 1.34 cc. (calculated at 0° C. and 760 mm. mercury).

According to BOHR, the facts are different. He differentiates between four oxyhæmoglobins, according to the quantity of oxygen which they absorb, namely

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[1] Hoppe-Seyler, Med. chem. Untersuch., 370; Jaquet, Zeitschr. f. physiol. Chem., **14**, 296; Kossel, *ibid*,. **2**, 150; Zinoffsky, *ibid*., **10**; Hüfner, Beitr. z. Physiol., Festschr. f. C. Ludwig, 1887, 74-81, Journ. f. prakt. Chem. (N. F.), **22**; Otto, Zeitschr. f. physiol. Chem., **7**; Inoko, *ibid*., **18**; Abderhalden and Medigreceanu, *ibid*., **59**; Hüfner and Jaquet, Arch. f. (Anat. u.) Physiol., 1894; E. Butterfield, Zeitschr. f. physiol. Chem., **62**.

[2] Barcroft and Hill, Journ. of Physiol. **39**; Hüfner and Gansser, Arch. f. (Anat. u.) Physiol., 1907.

[3] Bohr, "Sur les combinaisons de l'hémoglobine avec l'oxygène," Extrait du Bulletin de l'Académie Royale Danoise des sciences, 1890; also Centralbl. f. Physiol. 1890, 249. Hoppe-Seyler, Zeitschr. f. physiol. Chem., **2**; Hüfner, Arch. f. (Anat. u.) Physiol., 1894.

[4] Arch. f. (Anat. u.) physiol., 1901, Suppl.

α-, β-, γ- and δ-oxyhæmoglobin, all having the same absorption-spectrum, and 1 gram combining with respectively 0.4, 0.8, 1.7, and 2.7 cc. oxygen at the temperature of the room and with an oxygen pressure of 150 mm. mercury. The γ-oxyhæmoglobin is the ordinary one obtained by the customary method of preparation. BOHR designates as α-oxyhæmoglobin the crystallin powder obtained by drying γ-oxyhæmoglobin in the air. On dissolving α-oxyhæmoglobin in water it is converted into β-oxyhæmoglobin without decomposition, and the quantity of iron is increased. On keeping a solution of γ-oxyhæmoglobin in a sealed tube it is transformed into δ-oxhyæmoglobin, although the exact conditions under which this change takes place are not known. According to HÜFNER [1] these are nothing but mixtures of genuine and partly decomposed hæmoglobins.

The ability of hæmoglobin to take up oxygen seems to be a function of the iron it contains, and when this is calculated as about 0.33–0.40 per cent, then 1 atom of iron in the hæmoglobin corresponds to about 2 atoms or 1 molecule of oxygen. By increasing the partial pressure as well as by increasing the quantities of oxygen, the hæmoglobin in solution takes up more oxygen, until it is completely saturated, when 1 molecule of hæmoglobin is combined with 1 molecule of oxygen. With reduced oxygen pressure a dissociation must naturally take place and oxygen is given off, and a re-formation of hæmoglobin takes place, and this makes it possible to expel completely the oxygen from an oxyhæmoglobin solution or blood by means of vacuum, or by passing an indifferent gas through the solution. The equilibrium between oxyhæmoglobin, hæmoglobin, and oxygen depends, therefore, according to HÜFNER, upon a mass action, corresponding to the formula $Hb+O_2 \rightleftarrows HbO_2$. BOHR [2] has arrived at the conclusion that not only a double dissociation takes place, in which a dissociation of the oxygen-iron combination in the oxyhæmoglobin occurs, but also a dissociation of the hæmoglobin into a ferruginous as well as into a non-ferruginous part. Correspondingly he has suggested another formula and hence the dissociation curves for oxyhæmoglobin given by HÜFNER and BOHR are different.

Important investigations have recently been carried out on this question by BARCROFT and his co-workers CAMIS and ROBERTS from which it follows that a generally valid dissociation curve cannot be given, as the curve direction is dependent upon the nature and concentration of the salts present in the solution. A hæmoglobin solution with the salts of the blood-corpuscles of the dog gives a dissociation curve of dog-blood while with the salts from human blood-corpuscles it gives a curve like human blood. In the presence of salts the dissociation follows BOHR's formula, and on the contrary while a salt-free hæmoglobin solution follows the oxygen combination according to the mass-action law of HÜFNER.

[1] Arch. f. (Anat. u.) physiol., 1894.

[2] Bohr, Centralbl. f. physiol., 17, pp. 682 and 688.

Thus far there does not seem to be any necessity for considering the gas combination in the blood and in hæmoglobin solutions to be adsorption processes as suggested by W. OSTWALD.[1]

That the gas combining ability of an isolated pure hæmoglobin cannot be compared with the gas combining ability of the so-called native hæmoglobin of the blood has been suggested by many experimenters. In this connection we must mention the observations of MANCHOT[2] who found that the combining ability of the blood for gases such as O_2, CO,NO,C_2H_4 could be increased at least to a certain limit by increasing the dilution so that at 8–10 times the dilution the combining power was close to the limit value of 2 mol. gas for each atom of iron.

The elucidation of these mentioned conditions is of the greatest importance, as the knowledge of the various conditions which influence the taking up and the giving up of oxygen by the hæmoglobin is of the greatest importance for our knowledge of the taking up of oxygen in the lungs and the giving up of the same to the tissues.

Oxyhæmoglobin which is generally considered as a weak acid, is according to GAMGEE,[3] dextrorotatory. The specific rotation for light of medium wave-length of C is $(\alpha)C$=about $+10°$, which corresponds also for carbon-monoxide hæmoglobin. The hæmoglobin is also, like carbon-monoxide hæmoglobin (COHb) and methæmoglobin (MHb), diamagnetic, while the hæmatin, which is richer in iron, is strongly magnetic (GAMGEE[4]). On passing an electric current through an oxyhæmoglobin solution, the pigment first separates unchanged at the anode in a colloidal but still soluble form, and is then gradually transferred to the cathode in the colloidal state (GAMGEE[5]). According to GAMGEE, the hæmoglobin probably exists in such a colloidal condition in the blood-corpuscles.

Oxyhæmoglobin has been obtained in crystals from several varieties of blood. These crystals are blood-red, transparent, silky, and may be 2–3 mm. long. The oxyhæmoglobin from squirrel's blood crystallizes in six-sided plates of the hexagonal system; the other varieties of blood yield needles, prisms, tetrahedra, or plates which belong to the rhombic system.[6] The quantity of water of crystallization varies between

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[1] Barcroft with Camis, Journ. of Physiol., **39**; with Roberts, *ibid.*; W. Ostwald, Kolloid-Zeitschr., **2**, cited in Maly's Jahresb., **38**, 187.

[2] Annal. d. Chem. u. Pharm., **370** and Zeitschr. f. physiol. Chem., **70**.

[3] Hofmeister's Beiträge, **4**.

[4] Proceedings of Roy. Society, **68**.

[5] *Ibid.*, **70**.

[6] The observation of Uhlik (Pflüger's Arch., **104**) that the hæmoglobin from horse-blood can also crystallize in hexagonal plates seems to be due to the fact that he had hæmoglobin and not oxyhæmoglobin.

3–10 per cent for the different oxyhæmoglobins. When completely dried at a low temperature over sulphuric acid the crystals may be heated to 110–115° C. without decomposition. At higher temperatures, somewhat above 160° C., they decompose, giving an odor of burned horn, and leave, after complete combustion, an ash consisting of oxide of iron. The oxyhæmoglobin crystals from difficultly crystallizable blood, for example from such as ox's, human, and pig's blood, are easily soluble in water. The oxyhæmoglobins from easily crystallizable blood, as from that of the horse, dog, squirrel, and guinea-pig, are soluble with difficulty in the order above given. The oxyhæmoglobin dissolves more easily in a very dilute solution of alkali carbonate than in pure water, and this solution may be kept. The presence of a little too much alkali causes the oxyhæmoglobin to decompose quickly. The crystals are insoluble in absolute alcohol without decolorization. According to NENCKI[1] it is converted into an isomeric or polymeric modification, called by him *parahæmoglobin*. Oxyhæmoglobin is insoluble in ether, chloroform, benzene, and carbon disulphide.

A solution of oxyhæmoglobin in water is precipitated by many metallic salts, but is not precipitated by sugar of lead or basic lead acetate. On heating the watery solution it decomposes at about 70° C., and splits off protein and hæmatin when sufficiently heated. It is also readily decomposed by acids, alkalies, and many metallic salts. It gives the ordinary reactions for proteins with those protein reagents which first decompose the oxyhæmoglobin with the splitting off of protein. Oxyhæmoglobin, like the other blood-pigments, has a direct oxidizing action upon tincture of guaiacum. It has, on the other hand, like all blood-pigments containing iron, the property of an "ozone transmitter" in that it turns tincture of guaiacum blue in the presence of reagents containing peroxide, such as old turpentine.

A sufficiently dilute solution of oxyhæmoglobin or arterial blood shows a spectrum with two absorption-bands between the FRAUNHOFER lines *D* and *E* (spectrum Plate 1). The one band, α, which is narrower but darker and sharper, lies on the line *D*; the other, broader, less defined and less dark band, β, lies at *E*. The middle of the first band corresponds to a wave-length $\lambda = 579$ and the second $\lambda = 542$. On dilution the band β first disappears. By increased concentration of the solution the two bands become broader, the space between them smaller or entirely obliterated, and at the same time the blue and violet part of the spectrum is darkened. Besides these two bands we can also observe

[1] Nencki and Sieber, Ber. d. d. chem. Gesellsch., 18. According to Krüger (see Biochem. Centralbl., I, 40, 463) hæmoglobin is somewhat changed by alcohol as well as by chloroform.

by the aid of special appliances (L. LEWIN, MIETHE, and STENGER) the band first described by SORET and then by GAMGEE in the ultra-violet portion. This violet band, $\lambda = 415$, is of importance in the detection of very small quantities of blood. While the two oxyhæmoglobin bands are still detectable in a dilution of 1:14,700 the violet band may be seen, according to LEWIN, MIETHE and STENGER[1] in a dilution of 1:40,000.

The observation of PIETTRE and VILA that so-called laky blood and oxyhæmoglobin solutions in thick layers also show a third band in the red ($\lambda = 634$) depends in all probability, as also claimed by VILLE and DERRIEN, upon a partial formation of methæmoglobin which according to ARON[2] exists preformed in all blood.

A great many methods have been proposed for the preparation of oxyhæmoglobin crystals, but in their chief features they all agree with the following one suggested by HOPPE-SEYLER: The washed blood-corpuscles (best those from the dog or the horse) are stirred with 2 vols. of water and then shaken with ether. After decanting the ether and allowing the ether which is retained by the blood solution to evaporate in an open dish in the air, cool the filtered blood solution to 0° C., add while stirring one-fourth vol. of alcohol also cooled, and allow to stand a few days at −5° to −10° C. The crystals which separate may be repeatedly recrystallized by dissolving in water of about 35° C., cooling, and adding cooled alcohol as above. Lastly, they are washed with cooled water containing alcohol (one-quarter vol. alcohol) and dried in vacuum at 0° C. or a lower temperature.[3]

For the preparation of oxyhæmoglobin crystals in small quantities from easily crystallizable blood, it is often sufficient to stir a drop of blood with a little water on a microscope slide and allow the mixture to evaporate so that the drop is surrounded by a dried ring. After covering with a cover-glass, the crystals gradually appear radiating from the ring. These crystals are formed more surely if the blood is first mixed with some water in a test-tube and shaken with ether, and a drop of the lower deep-colored liquid treated as above on the slide.

Hæmoglobin, also called REDUCED HÆMOGLOBIN OR PURPLE CRUORIN (STOKES[4]), occurs only in very small quantities in arterial blood, in larger quantities in venous blood, and is almost the only blood-coloring matter after asphyxiation.

Hæmoglobin is much more soluble than the oxyhæmoglobin, and it can therefore be obtained as crystals only with difficulty. These

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[1] Soret, cited in Maly's Jaresb., 8; Gamgee, Zeitschr. f. Biol., **34**; Lewin, Miethe and Stenger, Pflüger's Arch., **118**; Lewin and Miethe, *ibid.*, **121**.

[2] Piettre and Vila, Compt. Rend., **140**; Ville and Derrien, *ibid.*, **140**; Aron, Biochem. Zeitschr., **3**.

[3] In regard to the preparation of oxyhæmoglobin, see also Hoppe-Seyler-Thierfelder's Handbuch, 8. Aufl.; also the works cited in footnote 1, p. 278; also Schuurmanns-Stekhoven, Zeitschr. f. physiol. Chem., **33**, 296; see also Bohr, Skand. Arch. f. Physiol., **3**; J. Offringa, Bioch. Zeitschr., **28**.

[4] Philosophical Magazine, **28**, No. 190, Nov., 1864.

crystals are as a rule isomorphous with the corresponding oxyhæmoglobin crystals, but are darker, having a shade toward blue or purple, and are decidedly more pleochromatic. The hæmoglobin from horse-blood has also been obtained by UHLIK [1] in hexagonal plates. Its solutions in water are darker and more violet or purplish than solutions of oxyhæmoglobin of the same concentration. They absorb the blue and the violet rays of the spectrum in a less marked degree, but strongly absorb the rays lying between *C* and *D*. In proper dilution the solution shows a spectrum with one broad, not sharply defined band between *D* and *E*, whose darkest part corresponds to the wave-length $\lambda = 559$ (spectrum Plate, 2). This band does not lie in the middle between *D* and *E*, but is toward the red end of the spectrum, a little over the line *D*. This pigment also gives a band in the ultra-violet, $\lambda = 429$. A hæmoglobin solution actively absorbs oxygen from the air and is converted into an oxyhæmoglobin solution.

A solution of oxyhæmoglobin may be easily converted into a solution having the spectrum of hæmoglobin by means of a vacuum, by passing an indifferent gas through it, or by the addition of a reducing substance, as, for example, an ammoniacal ferrous-tartrate solution (STOKES' reduction liquid). If an oxyhæmoglobin solution or arterial blood is kept in a sealed tube, we observe a gradual consumption of oxygen and a reduction of the oxyhæmoglobin into hæmoglobin. If the solution has a proper concentration, a crystallization of hæmoglobin may occur in the tube at lower temperatures (HÜFNER [2]).

Methæmoglobin. This name has been given to a coloring-matter which is easily obtained from oxyhæmoglobin as a transformation product and which has been correspondingly found in transudates and cystic fluids containing blood, in urine in hæmaturia or hæmoglobinuria, and also in urine and blood on poisoning with potassium chlorate, amyl nitrite or alkali nitrite, and many other bodies.

Methæmoglobin does not contain any oxygen in molecular or dissociable combination, but still the oxygen seems to be of importance in the formation of methæmoglobin, because it is formed from oxyhæmoglobin and not from hæmoglobin in the absence of oxygen or oxidizing agents. If arterial blood be sealed up in a tube, it gradually consumes its oxygen and becomes venous, and by this absorption of oxygen a little methæmoglobin is formed. The same occurs on the addition of a small quantity of acid to the blood. By the spontaneous decomposition of blood some methæmoglobin is formed, and by the action of ozone, potassium permanganate, potassium ferricyanide, chlorates, nitrites,

[1] Pflüger's Arch., 104.

[2] Zeitschr. f. physiol. Chem., 4; see also Uhlik, l. c.

nitrobenzene, pyrogallol, pyrocatechin, acetanilide, and certain other bodies on the blood an abundant formation of methæmoglobin takes place. By the action of light, HASSELBACH,[1] especially by the use of rays having a wave-light below 310 $\mu\,\mu$, obtained methæmoglobin from oxyhæmoglobin, but not from hæmoglobin in the absence of oxygen, and by this behavior pure methæmoglobin can be prepared.

According to the investigations of HÜFNER, KÜLZ, and OTTO[2] methæmoglobin contains just as much oxygen as oxyhæmoglobin, but it is more strongly combined, a view which is accepted by most investigators. According to HÜFNER and v. ZEYNEK we can admit in the methæmoglobin formation of an expulsion of oxygen and a combination of two hydroxyl groups; methæmoglobin would then be $Hb\begin{matrix}OH\\OH\end{matrix}$. According to others, HOPPE-SEYLER, KÜSTER, LETSCH the methæmoglobin contains less oxygen than the oxyhæmoglobin and is HbO or HbOH. A methæmoglobin solution is converted into a hæmoglobin solution by reducing substances. The reaction taking place in the formation of methæmoglobin from oxyhæmoglobin by the action of potassium ferricyanide has been quantitatively followed by v. REINBOLD.[3] He found that one molecule of $K_3Fe(CN)_6$ was necessary to transform 1 molecule oxyhæmoglobin or to drive off 1 molecule of oxygen from the oxyhæmoglobin. The reaction takes place according to the equation:

$$Hb\begin{matrix}O\\|\\O\end{matrix} + K_3Fe(CN)_6 + H_2O = Hb.OH + K_3HFe(CN)_6 + O_2$$

and from his investigations he gives the formula Hb.OH to methæmoglobin, in correspondence to the views of KÜSTER.

According to HÜFNER and REINBOLD[4] 1 gram methæmoglobin can take up 2.685 cc. nitric oxide.

Methæmoglobin crystallizes, as first shown by HÜFNER and OTTO, in brownish-red needles, prisms, or six-sided plates. It dissolves easily in water; the solution has a brown color and becomes a beautiful red on the addition of alkali. The solution of the pure substance is not precipitated by basic lead acetate alone, but by basic lead acetate and

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[1] Bioch. Zeitschr., **19**.

[2] See Otto, Zeitschr. f. physiol. Chem., **7**; v. Zeynek, Arch. f. (Anat. u.) Physiol., 1899; Hüfner, *ibid.*

[3] Küster, Zeitschr. f. physiol. Chem., **66**; Letsche, *ibid.*, **80**; v. Reinbold, Zeitschr. f. physiol. Chem., **85**.

[4] Arch. f. (Anat. u.) Physiol., 1904, Suppl.

ammonia. The absorption-spectrum of a watery or acidified solution of methæmoglobin is, according to JÄDERHOLM and BERTIN-SANS, very similar to that of hæmatin in acid solution, but is easily distinguished from the latter since, on the addition of a little alkali and a reducing substance, the former passes over to the spectrum of reduced hæmoglobin, while a hæmatin solution under the same conditions gives the spectrum of an alkaline hæmochromogen solution (see below). According to ARAKI and DITTRICH, a neutral or faintly acid methæmoglobin solution shows only one characteristic band, α, between C and D, whose middle corresponds to about $\lambda = 634$. The two bands between D and E are only due to contamination with oxyhæmoglobin (MENZIES, LEWIN, MIETHE and STENGER. According to HASSELBACH'S [1] experience a pure neutral solution of methæmoglobin gives four absorption bands corresponding to a maxima $\lambda = 630$, 580, 540 and 500. Methæmoglobin in alkaline solution shows two absorption-bands which are like the two oxyhæmoglobin bands, but they differ from these in that the band β is stronger than α. By the side of the band α and united with it by a shadow lies a third fainter band between C and D, near to D. (Spectrum Plate, 4.)

The claims as to the action of sodium fluoride upon hæmoglobin and methæmoglobin are somewhat contradictory.[2]

Crystallized methæmoglobin may be easily obtained by treating a concentrated solution of oxyhæmoglobin with a sufficient quantity of concentrated potassium-ferricyanide solution to give the mixture a porter-brown color. After cooling to 0° C. add one-fourth vol. cooled alcohol and allow the mixture to stand a few days in the cold. The crystals may be easily purified by recrystallizing from water by the addition of alcohol. According to HASSELBACH this method ordinarily gives impure products while a pure preparation can be obtained by the action of light (see above).

Cyanmethæmoglobin (cyanhæmoglobin) is, according to HALDANE, identical with photomethæmoglobin (BOCK), which is produced by the influence of sunlight upon a methæmoglobin solution containing potassium ferricyanide. It was first carefully described by R. KOBERT and obtained in a crystalline form by v. ZEYNEK.[3] It is immediately formed in the cold by the action of a hydrocyanic-acid solution upon methæmoglobin, but is formed by its action upon oxy-

[1] Jäderholm, Zeitschr. f. Biol. **16**; Bertin-Sans, Comp. Rend., **106**; Araki, Zeitschr. f. physiol. Chem., **14**; Dittrich. Arch. f. exp. Path. u. Pharm., **29**; Menzies, Journ. of Physiol., **17**; Lewin and collaborators, footnote 1, page 282; Hasselbach, Bioch. Zeitschr., **19**, and Proceedings of the 7th Internat. Congr. of Appl. Chem., London, 1909. Important references on methæmoglobin are given by Otto, Pflüger's Arch., **81**.

[2] Piettre and Vila, Compt. Rend., **140**; Ville and Derrien, *ibid.*, **140**.

[3] Haldane, Journ. of Physiol., **25**; Bock, Skand. Arch. f. Physiol., **6**; Kobert, Pflüger's Arch., **82**; v. Zeynek, Zeitschr. f. physiol. Chem., **33**. See also Leers, Biochem. Zeitschr., **12**.

hæmoglobin only at the body temperature. The neutral or faintly alkaline solutions show a spectrum which is very similar to the hæmoglobin spectrum. The question as to a special cyanmethæmoglobin is still disputed.

Acid hæmoglobin is a coloring-matter produced by the action of very weak acids upon oxyhæmoglobin, which according to HARNACK [1] is not, as used to be admitted, identical with methæmoglobin.

Carbon-monoxide Hæmoglobin [2] is the molecular combination between 1 molecule of hæmoglobin and 1 molecule of CO, according to HÜFNER,[3] which contains 1.34 cc. of carbon monoxide (at 0° and 760 mm. Hg) for 1 gram hæmoglobin. This combination is stronger than the oxygen combination of hæmoglobin. The oxygen is for this reason easily driven out of oxyhæmoglobin by carbon monoxide, and this explains the poisonous action of this gas, which kills by the expulsion of the oxygen of the blood. In regard to the division of the blood-pigments between the carbon monoxide and oxygen under different partial pressures of both gases in the air, we must refer to the investigations of HÜFNER, DOUGLAS and HALDANE.[4]

The carbon monoxide can be driven out by a vacuum as well as by passing an indifferent gas, or oxygen, or nitric oxide, through the solution for a long time, and in these cases hæmoglobin, oxyhæmoglobin, or nitric-oxide hæmoglobin are formed. The carbon-monoxide is also expelled by potassium ferricyanide and methæmoglobin is formed (HALDANE [5]). The above-mentioned behavior found by MANCHOT for the absorption of oxygen, namely, that the amount of gas taken up increases with the dilution of the blood so that for every atom of iron 2 mol. of gas are absorbed applies also for the carbon-monoxide hæmoglobin as well as for the nitric-oxide hæmoglobin, which will be discussed further on.

Carbon-monoxide hæmoglobin is formed by saturating blood or a hæmoglobin solution with carbon monoxide, and may be obtained as crystals by the same means as oxyhæmoglobin. These crystals are isomorphous with the oxyhæmoglobin crystals, but are less soluble and more stable, and their bluish-red color is more marked. For the detec-

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[1] Zeitschr. f. physiol. Chem., **26**.

[2] In reference to carbon-monoxide hæmoglobin, see especially Hoppe-Seyler, Med.-chem. Untersuch., 201; Centralbl. f. d. med. Wissensch., 1864 and 1865; Zeitschr. f. physiol. Chem., 1 and 13.

[3] Arch. f. (Anat. u.) Physiol., 1894. On the dissociation constant of carbon-monoxide hæmoglobin, see *ibid.*, 1895. In regard to the contradictory statements of Saint-Martin and others and their disapproval, see Hüfner, Arch. f. (Anat. u.) Physiol., 1903.

[4] Hüfner, Arch. f. exp. Path. u. Pharm., 48; Douglas and Haldane, Journ. of Physiol., 44.

[5] Journ. of Physiol., 22.

tion of carbon-monoxide hæmoglobin, its absorption-spectrum is of the greatest importance. This spectrum shows two bands which are very similar to those of oxyhæmoglobin, but they occur more toward the violet part of the spectrum. The middle of the first band corresponds to $\lambda=570$, and the second to $\lambda=542$ (LEWIN, MIETHE and STENGER). These bands do not change noticeably on the addition of reducing substances; this constitutes an important difference between carbon-monoxide hæmoglobin and oxyhæmoglobin. If the blood contains oxy-hæmoglobin and carbon-monoxide hæmoglobin at the same time, we obtain on the addition of a reducing substance (ammoniacal ferro-tartrate solution) a mixed spectrum originating from the hæmoglobin and carbon-monoxide hæmoglobin. Carbon-monoxide hæmoglobin also gives a band in the violet $\lambda=416$.

A great many reactions have been suggested for the detection of carbon-monoxide hæmoglobin in medico-legal cases. A simple and at the same time a good one is HOPPE-SEYLER'S alkali test. The blood is treated with double its volume of caustic-soda solution of 1.3 sp. gr., by which ordinary blood is converted into a dingy brownish mass, which when spread out on porcelain is brown with a shade of green. Carbon-monoxide blood gives under the same conditions a red mass, which if spread out on porcelain shows a beautiful red color. Several modifications of this test have been proposed. Another very good reagent is tannic acid, which gives with dilute normal blood a brownish-green precipitate and with carbon-monoxide blood a pale crimson-red precipitate.[1]

As according to BOHR there are several oxyhæmoglobins, so also according to BOHR and BOCK,[2] there are several carbon-monoxide hæmoglobins, with different amounts of carbon monoxide. As hæmoglobin can unite with oxygen and carbon dioxide simultaneously, as shown by BOHR and TROUP, so also can it unite with carbon monoxide and carbon dioxide simultaneously and independently of each other.

Carbon-monoxide methæmoglobin has been prepared by WEIL and v. ANREP by the action of potassium permanganate on carbon-monoxide hæmoglobin, but this is contradicted by BERTIN-SANS and MOITESSIER.[3] **Sulphur methæmoglobin** is the name given by HOPPE-SEYLER to that coloring-matter which is formed by the action of sulphureted hydrogen upon oxyhæmoglobin and which is generally designated *sulphæmoglobin*. The solution has a greenish-red, dirty color, and shows two absorption-bands between *C* and *D*. This coloring-matter is claimed to be the greenish color seen on the surface of putrefying flesh. According to HARNACK the conditions are different when H_2S is passed through an oxygen-free solution of hæmoglobin (or carbon-monoxide hæmoglobin). The

[1] In regard to this test (as suggested by Kunkel) and others we refer to Kostin, Pflüger's Arch., 84, which contains a very excellent summary of the literature on the subject. See also de Domenicis, Chem. Centralbl., 1908, 2, p. 66.

[2] Centralbl. f. Physiol., 8, and Maly's Jahresber., 25.

[3] v. Anrep, Arch. f. (Anat. u.) Physiol., 1880; Sans and Moitessier, Compt. Rend., 118.

sulphæmoglobin thus formed shows one band in the red between *C* and *D*. According to CLARKE and HURTLEY [1] the formation of sulphæmoglobin takes place after the reduction to hæmoglobin.

Carbon-dioxide Hæmoglobin, *Carbohæmoglobin.* Hæmoglobin, according to BOHR and TORUP,[2] also forms a molecular combination with carbon dioxide whose spectrum is similar to that of hæmoglobin. According to BOHR there are three different carbohæmoglobins, namely, α-, β-, and γ-carbohæmoglobin, in which 1 gram combines with respectively 1.5, 3, and 6 cc. CO_2 (measured at 0° C. and 760 mm.) at 18° C. and a pressure of 60 mm. mercury. If a hæmoglobin solution is shaken with a mixture of oxygen and carbon dioxide, the hæmoglobin combines loosely with the oxygen as well as with the carbon dioxide, independently of each other, just as if each gas existed alone (BOHR). He considers that the two gases are combined with different parts of the hæmoglobin, that is, the oxygen with the pigment nucleus and the carbon dioxide with the protein component. Attention must be called to the fact that, as observed by TORUP, hæmoglobin is in part readily decomposed by the carbon dioxide with the splitting off of some protein.

Nitric-oxide Hæmoglobin is also a crystalline molecular combination which is even stronger than the carbon-monoxide hæmoglobin. Its solution shows two absorption-bands, which are paler and less sharp than the carbon-monoxide hæmoglobin bands, and they do not disappear on the addition of reducing bodies. Hæmoglobin also forms a molecular combination with acetylene and ethylene.

Hæmorrhodin is the name given by LEHMANN to a beautiful red pigment soluble in alcohol and ether, which is extracted from meat and meat products by boiling alcohol and which seems to be produced by the action of small amounts of nitrites. Another pigment isolated by LEWIN [3] from the blood of animals poisoned by phenylhydrazine, has been called *hæmoverdin.* By heating a solution of blood-pigment treated with caustic potash and mixed with alcohol to 60° C. we obtain, according to v. KLAVEREN, a pigment which he calls *kathæmoglobin*, but called by ARNOLD,[4] who first obtained it, *neutral hæmatin*, which is produced by the splitting off of a ferruginous complex. This pigment still contains protein, but is poorer in iron than the hæmoglobin or methæmoglobin and probably forms an intermediary product in the conversion of the above into hæmatin.

Decomposition products of the blood-pigments. By its decomposition, hæmoglobin yields, as previously stated, a *protein* which has been

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[1] Hoppe-Seyler, Med.-chem. Untersuch., 151. See Araki, Zeitschr. f. physiol. Chem., **14**; Harnack, l. c.; Clarke and Hurtley, Journ. of Physiol., **36**.

[2] Bohr, Extrait, du Bull. de l'Acad. Danoise, 1890; Centralbl. f. Physiol., **4 and 17**; Torup, Maly's Jahresber., **17**.

[3] K. B. Lehmann, Sitzungsber. d. phys.-med. Gesellsch. Würzburg, 1899; **Lewin,** Compt. Rend., **133**.

[4] v. Klaveren, Zeitschr. f. physiol. Chem., **33**; Arnold, *ibid*, **29**.

called *globin* (PREYER, SCHULZ), and a ferruginous *pigment* as chief products. According to LAWROW 94.09 per cent protein, 4.47 per cent hæmatin, and 1.44 per cent other bodies are produced in this decomposition. The globin, which was isolated and studied by SCHULZ,[1] differs from most other proteins by containing a high amount of carbon, 54.97 per cent., with 16.98 per cent of nitrogen. It is insoluble in water, but very easily soluble in acids or alkalies. It is not dissolved by ammonia in the presence of ammonium chloride. Nitric acid precipitates it in the cold, but not when warm. It may be coagulated by heat, but the coagulum is readily soluble in acids. Because of these reactions it is considered as a histone by SCHULZ.

On hydrolytic cleavage globin (from horse-blood) yields, according to ABDERHALDEN,[2] the ordinary cleavage products of the proteins and especially leucine, 29 per cent. It is also important to call attention to the large amount of histidine, 10.96 per cent, while the quantities of arginine and lysine were only 5.42 and 4.28 per cent respectively.

The pigment split off is different, depending upon the conditions under which the cleavage takes place. If the decomposition takes place in the absence of oxygen, a coloring-matter is obtained which is called by HOPPE-SEYLER *hæmochromogen*, by other investigators (STOKES) *reduced hæmatin*. In the presence of oxygen, hæmochromogen is quickly oxidized to hæmatin, and there is therefore obtained in this case *hæmatin* as a colored decomposition product. As hæmochromogen is easily converted by oxygen into hæmatin, so this latter may be reconverted into hæmochromogen by reducing substances.

Hæmochromogen was discovered by HOPPE-SEYLER.[3] It is, according to HOPPE-SEYLER, the colored atomic group of hæmoglobin and of its combinations with gases, and this atomic group is combined with proteins in the pigment. The characteristic absorption of light depends on the hæmochromogen, and it is also this atomic group which binds, in the oxyhæmoglobin, 1 molecule of oxygen and, in the carbon-monoxide hæmoglobin, 1 molecule of carbon monoxide with 1 atom of iron. Hæmochromogen is produced in an alkaline solution of hæmatin by the action of reducing bodies. By the reduction of hæmatin in alcoholic ammoniacal solution by means of hydrazine v. ZEYNEK[4] was able to obtain the solid brownish-red ammonia combination. A crystalline combination between pyridine and hæmochromogen can be obtained according to KALMUS

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[1] Lawrow, *ibid.*, 26; Schulz, *ibid.*, 24; Preyer, Die Blutkristalle, Jena, 1871.
[2] Zeitschr. f. physiol. Chem., 37; with Baumann, *ibid.*, 51.
[3] *Ibid.*, 18.
[4] Zeitschr. f. physiol., Chem., 25.

and v. ZEYNEK[1] from hæmoglobin and pyridine by boiling, or from hæmatin and hæmin and pyridine after the addition of hydrazin-hydrate.

Hæmochromogen also combines, as HOPPE-SEYLER first showed, with carbon monoxide. This compound, which in aqueous solution gives a spectrum similar to oxyhæmoglobin, has been obtained by PREGL[2] in the solid condition as a deep-violet powder which is insoluble in absolute alcohol. In opposition to hæmoglobin the hæmochromogen combines with oxygen more firmly than with carbon monoxide. The assumption of HOPPE-SEYLER, that this compound is a combination of 1 molecule hæmochromogen and therefore contains 1 molecule carbon monoxide for 1 molecule of iron has been experimentally substantiated by HÜFNER and KÜSTER and by PREGL.[3]

An alkaline hæmochromogen solution has a beautiful cherry-red color. It shows two absorption-bands, first described by STOKES (spectrum Plate, 6), one of which is dark and whose center corresponds to $\lambda = 556.4$ between *D* and *E*, and a second broader band, less dark, which covers the FRAUNHOFER lines *E* and *b*. The middle of this band corresponds to $\lambda = 526$ to 530 according to LEWIN, MIETHE and STENGER. In acid solution hæmochromogen shows four bands, which, according to JÄDERHOLM,[4] depend on a mixture of hæmochromogen and hæmatoporphyrin (see below), this last formed by a partial decomposition resulting from the action of the acid.

MILROY,[5] from an alcoholic solution of hæmatin containing oxalic acid, after driving out the air by means of hydrogen gas, gradually obtained an acid solution of reduced hæmatin (hæmochromogen) by means of zinc dust. This solution showed one absorption-band between *D* and *E*.

Hæmochromogen may be obtained as crystals by the action of caustic soda on hæmoglobin at 100° C. in the absence of oxygen (HOPPE-SEYLER). By the decomposition of hæmoglobin by acids (of course in the absence of air) we obtain hæmochromogen contaminated with a little hæmatoporphyrin. An alkaline hæmochromogen solution is easily obtained by the action of a reducing substance (STOKES' reduction liquid) on an alkaline hæmatin solution. An ammoniacal solution of hæmatin on reduction with hydrazine yields hæmochromogen very easily. An alcoholic, alkaline hydrazine solution is also recommended by RIEGLER[6] as a reagent for blood-pigments, converting them into hæmochromogen.

Hæmatin, also called OXYHÆMATIN, is sometimes found in old transudates. It is formed by the action of the gastric or pancreatic juices on

[1] E. Kalmus, Zeitschr. f. Chem., **70**; v. Zeynek, *ibid.*, **70**.
[2] *Ibid.*, **44**.
[3] Hüfner and Küster, Arch. f. (Anat. u.) Physiol., 1904, Suppl. Pregl, l. c.
[4] Nord. Med. Arkiv., **16**.
[5] Journ. of Physiol., **32**.
[6] Zeitschr. f. anal. Chem., **43**.

oxyhæmoglobin, and is, therefore, found in the feces after hemorrhage in the intestinal canal, and also after a meat diet and food rich in blood. It is stated that hæmatin may occur in urine after poisoning with arseniureted hydrogen. As shown above, the hæmatin is formed by the decomposition of oxyhæmoglobin, or at least of hæmoglobin, in the presence of oxygen.

The views in regard to the composition of hæmatin are rather contradictory, which seems to be due to the fact that the substance hæmin (see below), from which the formula of hæmatin is derived, has a somewhat different composition, dependent upon various conditions. According to HOPPE-SEYLER hæmatin has the formula $C_{34}H_{34}N_4FeO_5$, and from the recent investigations upon hæmin, which will be mentioned below, this formula seems to be now generally accepted. According to this formula 1 atom of iron occurs with every 4 atoms of nitrogen. According to CLOËTTA, and also ROSENFELD,[1] hæmatin has the formula $C_{30}H_{34}N_3FeO_3$, with 1 atom of iron for every 3 atoms of nitrogen.

v. ZEYNEK has prepared a hæmatin by the digestion of an oxyhæmoglobin solution with pepsin-hydrochloric acid, from which he then prepared hæmin. As this hæmatin of v. ZEYNEK was readily convertible into hæmin, and while the ordinarily prepared hæmatin from hæmin cannot be retransformed into hæmin, KÜSTER considers that these two forms of hæmatin are not identical. The first he calls α-hæmatin and the ordinary which is a polymeric body, he calls β-hæmatin. That a retransformation of hæmin is possible from ordinary hæmatin is still admitted by PILOTY and ELLINGER.[2]

Hæmatin contains at least three hydroxyl groups, one of which acts as hydroxyl ion and seems to be united with the iron, and is replaced in the hæmin formation (see below) by the chlorine. By means of the two others, salts with metals as well as alkyl derivatives may be formed, which latter (as hæmin derivatives) have been especially studied by NENCKI and ZALESKI and KÜSTER.[3] Hæmatin dissolves in concentrated sulphuric acid and is converted into hæmatoporphyrin, with the splitting off of iron. On heating dry hæmatin it yields an abundance of pyrrol. The products produced on the oxidation and reduction of hæmatin and the question as to the constitution of hæmatin will be discussed in connection with hæmatoporphyrin.

Hæmatin is amorphous, dark brown or bluish-black. It may be heated to 180° C. without decomposition; on burning it leaves a residue

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[1] Hoppe-Seyler, Med.-chem. Untersuch., p. 525; Cloetta, Arch. f. exp. Path. u. Pharm., **26**; Rosenfeld, *ibid.*, **40**.

[2] v. Zeynek, Zeitschr. f. physiol. Chem., **30** and **49**; Küster, *ibid.*, **66** and Ber. d. d. chem. Gesellsch., **43**; Piloty, Annal. d. Chem. u. Pharm., **377**; Eppinger, Unters. über den Blutfarbstoff. Dissert. München, 1907.

[3] Nencki and Zaleski, Zeitschr. f. physiol. Chem., **30**; Küster, Ber. d. d. Chem. Gesellsch. **43** and **45**, and Zeitschr. f. physiol. Chem., **82**.

consisting of iron oxide. It is insoluble in water, dilute acids, alcohol, ether, and chloroform, but it dissolves slightly in warm glacial acetic acid. Hæmatin dissolves in acidified alcohol or ether. It easily dissolves in alkalies, even when very dilute. The alkaline solutions are dichroic; in thick layers they appear red by transmitted light and in thin layers greenish. The alkaline solutions are precipitated by lime- and baryta-water, as also by solutions of neutral salts of the alkaline earths. The acid solutions are always brown.

An acid hæmatin solution (spectrum Plate, 4), absorbs the red part of the spectrum only slightly and the violet parts strongly. The solution shows a rather sharply defined band between *C* and *D*, whose position may change with the variety of acid used as a solvent. Between *D* and *F* a second, much broader, less sharply defined band occurs, which by proper dilution of the liquid is converted into two bands. The one between *b* and *F*, lying near *F*, is darker and broader; the other, between *D* and *E*, lying near *E*, is lighter and narrower. Also by proper dilution a fourth very faint band is observed between *D* and *E*, lying near *D*. Hæmatin may thus in acid solution show four absorption-bands; ordinarily one sees, distinctly, only the bands between *C* and *D* and the broad, dark band—or the two bands—between *D* and *F*. In alkaline solution, hæmatin (spectrum Plate, 5), shows a broad absorption-band, which lies in greatest part between *C* and *D*, but reaches a little over the line *D* toward the right in the space between *D* and *E*. As the position of the hæmatin bands in the spectrum is quite variable, the exact wave-lengths corresponding thereto cannot be given exactly.

Hæmin, Hæmin Crystals, or Teichmann's Crystals. Hæmin is formed, as generally admitted, by the replacement of an HO group by chlorine in the hæmatin, and is the starting point in the preparation of the latter.

The statements as to the composition of hæmin differ quite considerably, and various hæmins have been accepted, which is partly due to the fact, as first shown by Nencki and Zaleski, that hæmin combines with acid and alkyl radicals and can also give addition products with other bodies. Thus for example the methylhæmins, carefully studied by Küster, especially monomethylhæmin, is produced in the preparation of hæmin according to Mörner's method (see below) by means of methyl alcohol. These behaviors have been further explained by the work of numerous investigators, especially by Küster, and most investigators generally admit that only one hæmin exists whose general formula is $C_{34}H_{32}O_4N_4FeCl$. According to Piloty the formula is $C_{34}H_{32}O_4N_4FeCl$ while Pietttre and Vila [1] deny this formula and claim to have

[1] Nencki and Zaleski, Zeitschr. f. physiol. Chem., **30**; Nencki and Sieber, Arch. f. exp. Path. u. Pharm., **18** and **20**, and Ber. d. d. chem. Gesellsch., **18**; Schalfejeff

prepared a hæmin free from chlorine, from pure crystalline oxy-hæmoglobin.

Hæmin crystals form, in large masses, a bluish-black powder, but are so small that they can be seen only by aid of the microscope. They consist of dark-brown or nearly brownish-black long, rhombic, or spool-like crystals, isolated or grouped as crosses, rosettes, or stellar forms. Cubical crystals may also occur, according to CLOËTTA. They are insoluble in water, dilute acids at the normal temperature, alcohol, ether, and chloroform. They are slightly soluble in glacial acetic acid with heat. They dissolve in acidified alcohol, as also in dilute caustic alkalies or carbonates; and in the last case they form, besides alkali chlorides, soluble hæmatin alkali, from which the hæmatin may be precipitated by an acid. As shown by PILOTY and EPPINGER and then also by v. SIEWERT,[1] crystalline hæmin can be reobtained from the hæmatin.

On shaking with cold aniline and treating first with acetic acid and then with ether, KÜSTER obtained a product, dehydrochloride hæmin, which was poor in the elements of hydrochloric acid, and which again took up HCl and was converted into hæmin. By the action of boiling aniline, hydrogen is driven out and a combination with aniline, without loss of iron, takes place.

The principle of the preparation of hæmin crystals in large quantities is as follows: The washed sediment from the blood-corpuscles is coagulated with alcohol or by boiling after dilution with water and the careful addition of acid. The strongly pressed but not dry mass is rubbed with 90–95 per cent alcohol which has been previously treated with oxalic acid or ½–1 per cent concentrated sulphuric acid, and this is allowed to stand several hours at the temperature of the room. The filtrate is warmed to about 70° C., treated with hydrochloric acid (for each liter of filtrate add 10 cc. 25 per cent hydrochloric acid diluted with alcohol—MÖRNER), and allowed to stand in the cold. The crystals, which separate in one or two days, are first washed with alcohol and then with water. On dissolving the hæmin in chloroform containing quinine and treating the filtrate with alcoholic hydrochloric or acetic acid we can recrystallize the hæmin according to SCHALFEJEFF. By adding glacial acetic acid saturated with salt to a solution of hæmatin in chloroform containing quinine PILOTY and EPPINGER obtained crystalline hæmin. For particulars as to the various methods of preparation and purification we refer the reader to the above-cited works of NENCKI and SIEBER, MÖRNER, NENCKI and ZALESKI (SCHALFEJEFF), and especially to KÜSTER.[2]

Hæmatin is obtained on dissolving the hæmin crystals in very dilute caustic alkali and precipitating with an acid.

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with Nencki and Zaleski, l. c.; Bialobrzeski, Arch. des scienc. biol. de St. Pétersbourg, 5; K. Mörner, Nord. Med. Arkiv. Festband, 1897, Nos. 1 and 26, and Zeitschr. f. physiol. Chem., 41; Zaleski, *ibid.*, 37; Hetper and Marchlewski, *ibid.*, 41 and 42; Küster, *ibid.*, 40 and 82 and footnote 1, page 292; Piettre and Vila, Compt. Rend., 141, p. 734; Piloty, l. c.

[1] Piloty and Eppinger, l. c.; v. Siewert, Arch. f. exp. Path. u. Pharm., 58.

[2] Küster, Zeitschr., f. physiol. Chem., 40.

In preparing hæmin crystals in small quantities proceed in the following manner: The blood is dried after the addition of a small quantity of common salt, or the dried blood may be rubbed with a trace of the same. The dry powder is placed on a microscope slide, moistened with glacial acetic acid, and then covered with the cover-glass. Add, by means of a glass rod, more glacial acetic acid by applying the drop at the edge of the cover-glass until the space between the slide and the cover-glass is full. Now warm over a very small flame, with the precaution that the acetic acid does not boil and pass with the powder from under the cover-glass. If no crystals appear after the first warming and cooling, warm again, and if necessary add some more acetic acid. After cooling, if the experiment has been properly performed, a number of dark-brown or nearly black hæmin crystals of varying forms will be seen.

In regard to the preparation and properties of the iodine-, bromine-, and acetone-hæmin we refer to the work of STRZYZOWSKI, MERUNOWICZ and ZALESKI.[1]

By the action of acids upon hæmochromogen, hæmatin, or hæmin, a new iron-free pigment, which was first closely studied by HOPPE-SEYLER and called *hæmatoporphyrin*, is produced. According to the method of preparation, hæmatoporphyrins having different solubilities, and whose relation to each other is not perfectly clear, are produced, but all show the same characteristic absorption-spectrum. The best-studied hæmatoporphyrin is the one obtained according to NENCKI and SIEBER'S method, by the action of glacial acetic acid saturated with hydrobromic acid upon hæmin crystals, best at the temperature of the body (NENCKI and ZALESKI). Another porphyrin is the *mesoporphyrin* obtained by NENCKI and ZALESKI[2] by the reduction of hæmin in glacial acetic acid by hydriodic acid and iodophosphonium.

Hæmatoporphyrin, $C_{34}H_{38}N_4O_6$, which, according to recent molecular weight determinations must perhaps be doubled (PILOTY) occurs according to MAC MUNN[3] as a physiological pigment in certain animals. A porphyrin occurs, as shown by GARROD and SAILLET, as a normal constituent in human urine, although only as traces and it has also been observed several times in large amounts in the urine after the use of sulphonal (see Chapter XIV). This urine porphyrin is generally considered as hæmatoporphyrin.

In the production of hæmatoporphyrin from hæmin or hæmatin the iron is split off. Opinions are not unanimous in regard to this process. According

[1] Strzyzowski, Therap. Monatsh., 1901 and 1902; Merunowicz and Zaleski, Bull. de l'Acad. d. Scienc. de Cracovie, 1907.

[2] Hoppe-Seyler, Med.-chem. Untersuch., 528; Nencki and Sieber, Monatshefte f. Chem., **9**, and Arch. f. exp. Path. u. Pharm., **18**, **20**, and **24**; Nencki and Zaleski, Zeitschr. f. physiol. Chem., **30**.

[3] Piloty, Annal. d. Chem. u. Pharm., **388**; MacMunn, Journ. of Physiol., **7**.

to PILOTY two carboxyl groups are formed with the taking up of water and these occur to a certain extent latent in lactam combination in the hæmin. According to KÜSTER,[1] who admits of two already formed carboxyls in the hæmin, two hydroxyls are produced secondarily in the hæmatoporphyrin, in that (by the action of the glacial acetic acid and hydrobromic acid) primarily an attachment of hydrobromic acid takes place and then from this as intermediary product, by the action of water, bromine is split off and is replaced by hydroxyl. In the formation of mesoporphyrin the procedure is still different because among others, mesoporphyrin contains 2 oxygen atoms less than the hæmatoporphyrin.

On the gentle reduction of hæmin with glacial acetic acid, hydriodic acid and red phosphorus, PILOTY and FINK obtained besides mesoporphyrin a second body, *phonoporphyrin*, which differs from the mesoporphyrin by containing more oxygen, a brown color and almost complete insolubility in dilute hydrochloric acid. It is not reduced to mesoporphyrin by hydriodic acid but yields hæmatinic acid and methyl-ethyl maleic imide on oxidation with chromic acid. They obtained no other cleavage products from hæmin under the above mentioned experimental conditions. The two porphyrins were produced in about equal quantities and they formed about 90 per cent of the calculated cleavage products. They each represent one-half of the hæmin, whose formula corresponds to $C_{68}H_{64}N_8O_8Fe_2Cl_2$ which must be doubled. As these two porphyrins yield methyl ethylmaleic imide, while this is not the case with either the hæmin or the hæmatoporphyrin, it is believed that both are combined together in the hæmin or hæmatoporphyrin with that part of their molecules which allow of the maleic imide formation.

By the action of glacial acetic acid, and hydriodic acid upon hæmin in the cold (room temperature) in the presence of iodophosphonium, H. FISCHER and BARTHOLOMÄUS have obtained a beautiful crystalline, colorless product, *porphyrinogen*, whose formation and behavior have been further studied by RÖSE.[2] Porphyrinogen, $C_{34}H_{42}N_4O_4$ is formed from hæmin, the meso- and the hæmatoporphyrin in acid, and from the meso- or hæmatoporphyrin also in alkaline reduction. Porphyrinogen can be transformed into mesoporphyrin by oxidative action of various kinds. Like the latter it yields hæmatinic acid as well as methyl ethyl maleic imide as oxidation products.

Hæmatoporphyrin is closely related to the bile pigment bilirubin (see Chapter VII) and also stands in close relation with the urinary pigment, urobilin. By action of reducing substances several investigators (HOPPE-SEYLER, NENCKI and SIEBER, LE NOBEL, MACMUNN and others) have obtained pigments similar to urobilin, and by experiments with rabbits, NENCKI and ROTSCHY[3] have proved that hæmatoporphyrin introduced into the animal body may in part be transformed into a urobilin substance.

In connection with the question of the behavior of hæmatoporphyrin in the animal body, the poisonous action of this body, as discovered by HAUSMANN, and which manifests itself as a photobiological sensibilisation, is of interest. HAUSMANN has found that white mice that have had hæmatoporphyrin injected

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[1] Piloty, l. c.; Küster, Ber. d. d. chem. Gesellsch., **45**.

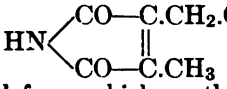
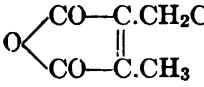
[2] Piloty and Fink, Ber. d. d. chem. Gesellsch., **46**, 2021; H. Fischer and Bartholomäus, *ibid.*, **46**, 511; Röse, Zeitschr. f. physiol. Chem., **84**.

[3] Hoppe-Seyler, Med. Chem. Unters. p. 533; Le Nobel, Pflüger's Arch., **40**; Nencki and Sieber, l. c.; MacMunn, Proc. Roy. Soc., **80**, and Journ. of Physiol., **10**; Nencki and Rotschy, Monatsh. f. chem., **10**.

subcutaneously and then exposed to bright light die very quickly with characteristic symptoms, while control animals kept in the dark show no symptoms of disease. H. FISCHER and MEYER-BETZ [1] have also shown that in this regard a certain difference exists between the hæmatoporphyrin and the mesoporphyrin. The perfectly pure crystalline mesoporphyrin does not show the photobiological action which occurs with crystalline hæmatoporphyrin.

Of especial interest is the close relationship of the hæmatoporphyrin to certain chlorophyll derivatives, especially to *phylloporphyrin*, $C_{32}H_{36}N_4O_2$. Phylloporphyrin is similar to the above-mentioned mesoporphyrin, $C_{34}H_{38}N_4O_4$, and the absorption spectrum of the bromporphyrins, bromphylloporphyrin and brommesoporphyrin as prepared by SCHUNCK and MARCHLEWSKI, seems to be almost identical. Just as from mesoporphyrin, with sodium chloride, glacial acetic acid and an iron salt we can regenerate a product very similar to hæmin (ZALESKI) so MARCHLEWSKI [2] has been able under similar conditions to prepare from phylloporphyrin a pigment, *phyllohæmin*, which contained iron and was similar to hæmin. A comparisen of the cleavage products gives still more conclusive and important proofs of the close relationship of the blood and leaf pigments.

We have important investigations of KÜSTER,[3] PILOTY, WILLSTÄTTER, H. FISCHER [4] and their collaborators upon the constitution of hæmin and hæmatoporphyrin. The constitution of chlorophyll has been explained by the pioneering researches of WILLSTÄTTER.

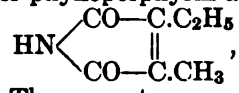
On the oxidation of hæmatin in glacial acetic acid by potassium dichromate or chromium trioxide KÜSTER obtained the imide of the tribasic *hæmatinic acid*,  , which is a derivative of maleic acid, and from which methylethylmaleic acid anhydride,  , can be readily obtained. The same hæmatinic

[1] Hausmann, Bioch. Zeitschr., **30**; Hans Fischer and Meyer-Betz. Zeitschr. f. physiol. Chem., **82**.

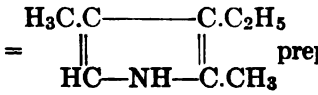
[2] The pertinent literature will be found in L. Marchlewski, Die Chemie der Chlorophylle und ihre Beziehung zur Chemie des Blutfarbstoffes, 1909 and Ber. d. d. chem. Gesellsch., **45**.

[3] Beiträge zur Kenntnis. des Hämatins, Tubingen, 1896; Ber. d. d. chem. Gesellsch., **27**, **30**, **32**, **35**, **43**, and **45**, Annal. d. Chem. u. Pharm., **315**, and Zeitschr. f. physiol. Chem., **28**, **40**, **44**, **54**, **55**, **61**, **62**, and **82**.

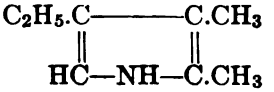
[4] The work of Piloty and collaborators may be found in Annal. d. Chem. u. Pharm., **366**, **377**, **388**, **390**, and **392** and Ber. d. d. chem. Gesellsch., **42**, **43**, and **45**. In regard to the work of Willstätter and the literature on chlorophyll (to 1911) see Willstätter in Abderhalden's Bioch. Handlexicon, Bd. VI and Annal. d. Chem. u. Pharm., **378**, **380**, **382**, and **385**. Hans Fischer and collaborators, Zeitschr. f. physiol. Chem., **82**, and footnote 2, p. 297, and the literature on bilirubin Chapter VII.

acid imide, which also hæmato- and mesoporphyrin give, were obtained by MARCHLEWSKI on the oxidation of phylloporphyrin and by WILLSTÄTTER, besides *methylethylmaleic imide*,  , on the oxidation of certain chlorophyll derivatives. The same two products were obtained by KÜSTER [1] on the oxidation of mesoporphyrin while hæmin and hæmatoporphyrin gave no methylethylmaleic imide.

It has been known for a long time that hæmin and hæmatoporphyrin gave an abundance of pyrrol on heating, and that phylloporphyrin has a similar behavior was first shown by SCHUNCK and MARCHLEWSKI. That at least one pyrrol, of the pyrrol mixture, the so-called *hæmopyrrol* is common to both the blood and leaf pigments has been shown by the investigations of MARCHLEWSKI and his collaborators, and from hæmopyrrol KÜSTER was the first to obtain methylethylmaleic imide on oxidation, showing that hæmopyrrol was probably a dimethylethylpyrrol. This behavior has been further developed by the investigations of PILOTY and WILLSTÄTTER on the reduction products of the blood and leaf pigments and by H. FISCHER and BARTHOLOMÄUS [2] on the substituted pyrrols.

WILLSTÄTTER obtained a pyrrol mixture from hæmin and hæmatoporphyrin, as well as from chlorophyll derivatives, by reduction, from which he isolated three different pyrrols. The first, which he calls *hæmopyrrol*, was perhaps not perfectly pure, consisted at least in great part of the *cryptopyrrol* (FISCHER and BARTHOLOMÄUS, *c*-hæmopyrrol of PILOTY and STOCK) which is identical with the 2, 4-dimethyl-3-ethyl pyrrol =  prepared synthetically by KNORR and HESS.[3]

The second hæmopyrrol, which he calls *isohæmopyrrol* (=hæmopyrrol of FISCHER and BARTHOLOMÄUS, B-hæmopyrrol of PILOTY and STOCK) is also a trisubstituted pyrrol, namely 2, 3-dimethyl-4-ethylpyrrol



These two dimethylethylpyrrols give with nitrous acid the correspond-

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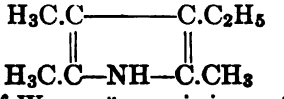
[1] Ber. d. d. chem. Gesellsch., **45**.

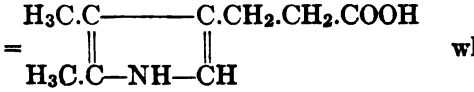
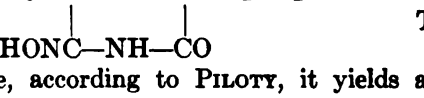
[2] Piloty, Annal. d. Chem. u. Pharm., **377**, **388**, **390**, and **392**; Willstätter and Asahina, *ibid.*, **385**. In this article will be found on pages 189 and 190 the references to the literature on the investigations of Marchlewski and others on hæmopyrrol. H. Fischer and Bartholomäus, Zeitschr. f. physiol. Chem., **77** and **80** and Ber. d. d. chem. Gesellsch., **45**.

[3] Piloty and Stock, Annal. d. Chem. u. Pharm., **392**; Knorr and Hess, Ber. d. d. chem. Gesellsch., **44** and **45**.

ing oxime of methylethylmaleic imide. The third pyrrol found by WILLSTÄTTER, which he calls *phyllopyrrol*, is a tetra-substituted pyrrol, namely, 2, 3, 5 trimethyl-4-ethylpyrrol = 

The statement that the hæmopyrrol of WILLSTÄTTER is in part derived from cryptopyrrol is not correct, and must be changed because of the investigations of PILOTY and STOCK, who find that the hæmopyrrol of WILLSTÄTTER (and ASAHINA) undoubtedly contains cryptopyrrol, but consists chiefly of the B-hæmopyrrol, consequently isohæmopyrrol. According to more recent investigations of PILOTY and STOCK [1] the hæmopyrrol question is even more complicated than was expected.

In the crude pyrrol obtained by the reduction of the blood pigments several other pyrrol bodies have been found, for example the *phonopyrrol* of PILOTY which has not been sufficiently explained. According to GRABOWSKI and MARCHLEWSKI [2] as well as to PILOTY and STOCK, the crude hæmopyrrol contains also disubstituted pyrrol, namely, β_1, β-methylethylpyrrol. On fusing hæmatoporphyrin or hæmatopyrrolidinic acid (see below) with caustic alkali we obtain, according to PILOTY, a mixture of pyrrols among which we will mention 2, 3-dimethylpyrrol , which has been studied by PILOTY and WILKE.[3]

By the reduction of hæmatoporphyrin and hæmin by various methods, PILOTY and co-workers have obtained, besides hæmopyrrol, several acids namely hæmatopyrrolidinic acid, phonopyrrolcarboxylic acid (isophonopyrrolcarboxylic acid) and xanthopyrrolcarboxylic acid. The hæmatopyrrolidinic acid seems from the most recent investigations not to be a unit substance. PILOTY obtained from it *phonopyrrolcarboxylic acid*, $C_9H_{13}NO_2$ =  which on treatment with nitrous acid lost a methyl group and was converted into the oxime of hæmatinic acid,  The acid received this name because, according to PILOTY, it yields a special dimethylethylpyrrol, called phonopyrrol by him. The question as to the nature of xanthopyrrolcarboxylic acid and its occurrence has not been

[1] Piloty and Stock, Annal. d. Chem. u. Pharm., **392** and Ber. d. d. chem. Gesellsch., **46**, 1008.

[2] Grabowski and Marchlewski, Zeitschr. f. physiol. Chem., **81**.

[3] Piloty and Wilke, Ber. d. d. chem. Gesellsch., **45**.

answered; still there does not seem to be any doubt that there exists an isophonopyrrolcarboxylic acid, which can be obtained from the blood as from the bile pigments. From the mixture of acid cleavage products obtained by the reduction of hæmin with hydriodic acid, and glacial acetic acid PILOTY and DORMANN [1] have obtained as well characterized products, phonopyrrolcarboxylic acid and isophonopyrrolcarboxylic acid and also *xanthopyrrolcarboxylic* acid, $C_{10}H_{15}NO_2$ and they consider the existence of this acid as positively proved. The melting-point of the crystalline acid was 108°, the picrate 143°, and the oxime 208°. The corresponding values for isophonopyrrolcarboxylic acid was 122°, 146° and 210° respectively. An isomeric xanthopyrrolcarboxylic acid, called D-phonopyrrolcarboxylic acid, seems also to occur.

It is extremely difficult to correlate the somewhat contradictory statements of the various authors in this subject and to draw quite positive conclusions from these statements. It is nevertheless positive that from the hæmopyrrol mixture the three pyrrols, cryptopyrrol, isohæmopyrrol and phyllopyrrol can be obtained and also that there are two hæmopyrrolcarboxylic acids (phonopyrrol- and isophonopyrrolcarboxylic acids), of which one possibly is related to the cryptopyrrol and the other to the isohæmopyrrol. On account of the uncertainty of the experimental foundation it is difficult to enter into a discussion of the variously proposed hypothetical constitutional formulæ for the derivatives of the blood pigments. The same is true for the disputed question as to the form of binding of the iron in hæmatin and in hæmin. It is generally admitted that the iron here is trivalent. The views are different in regard to the valence of the iron in hæmoglobin, namely, MANCHOT considers that hæmoglobin is a ferric combination while KÜSTER [2] on the contrary considers it a ferrous combination.

Hæmatoporphyrin gives with hydrochloric acid a compound which crystallizes in long brownish-red needles. If the solution in hydrochloric acid is nearly neutralized with caustic soda and then treated with sodium acetate, the pigment separates out as amorphous, brown flakes not readily soluble in amyl alcohol, ether, or chloroform, but readily soluble in ethyl alcohol, alkalies, and dilute mineral acids. The compound with sodium crystallizes as small tufts of brown crystals and several other salts of hæmatoporphyrin are known such as the methyl and ethyl esters. The acid alcoholic solutions have a beautiful purple color, which become violet-blue on the addition of large quantities of acid. The alkaline solution has a beautiful red color, especially when too much alkali is not present.

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[1] Piloty and Dormann, Ber. d. d. chem. Gesellsch., **46**.

[2] Manchot, Zeitschr. f. physiol. Chem., **70**; Küster, *ibid.*, **71**.

An alcoholic solution of hæmatoporphyrin, acidulated with hydrochloric or sulphuric acid, shows two absorption-bands (spectrum Plate, 7), one of which is fainter and narrower and lies between *C* and *D*, near *D*. The other is much darker, sharper, and broader, and lies midway between *D* and *E*. An absorption extends from these bands toward the red, terminating with a dark edge, which may be considered as a third band between the other two.

A dilute alkaline solution shows four bands, namely, a band between *C* and *D*; a second, broader band surrounding *D* and with the greater part between *D* and *E*; a third, between *D* and *E*, nearly at *E*; and lastly, a fourth, broad and dark band between *b* and *F*. On the addition of an alkaline zinc-chloride solution the spectrum changes more or less rapidly,[1] and finally a spectrum is obtained with only two bands, one of which surrounds *D* and the other lies between *D* and *E*. If an acid hæmatoporphyrin solution is shaken with chloroform, a part of the pigment is taken up by the chloroform, and this solution often shows a five-banded spectrum with two bands between *C* and *D*. The position of the hæmatoporphyrin bands in the spectrum differ with the various methods of preparation and other conditions, so that they do not correspond to the same wave length. These facts coincide well with the recent investigations of A. SCHULZ;[2] according to which the appearance of the spectrum is not only dependent upon the reaction but also upon the character of the solvent and the method of preparation.

In regard to the preparation of hæmatoporphyrin, see HOPPE-SEYLER-THIERFELDER'S Handbuch, 8. Aufl., and the works cited on page 294.

Mesoporphyrin, $C_{34}H_{38}N_4O_4$, has the same spectrum as hæmatoporphyrin. It has two oxygen atoms less, and further differs from it in that on oxidation it yields hæmatinic acid as well as methylethylmaleic imide, and does not show the above-mentioned biological action of hæmatoporphyrin.

Hæmatinogen is a ferruginous pigment so named by FREUND,[3] which he obtained by carefully extracting blood with alcohol containing hydrochloric acid. It is closely related to hæmatin, but is not sufficiently characteristic and is not considered as a cleavage product.

A question of great interest is whether it is possible to produce the blood-pigment from its cleavage products. In this respect certain recent investigations are interesting. ZALESKI obtained from mesoporphyrin hydrocloride dissolved in 80 per cent acetic acid saturated with NaCl and heated to 50°–70°, a hæmin-like pigment by the addition of a solution of iron in acetic acid, and this pigment had a spectrum in acid solution very similar to that of hæmatin, although not identical with it.

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[1] See Hammarsten, Skand. Arch. f. Physiol., 8, and Garrod, Journ. of Physiol., 18.

[2] Arch. f. (Anat. u.) Physiol., 1904, Suppl.

[3] Wien. klin. Wochenschr., 1903.

ZALESKI considers this pigment as a hydrogenized hæmin. A regeneration of hæmatin from hæmatoporphyrin has been performed by LAIDLAW. If hæmatoporphyrin is dissolved in dilute ammonia and warmed with STOKES' solution and hydrazine hydrate, iron is taken up again and hæmochromogen is produced, which is changed into hæmatin by shaking with air. According to HAM and BALEAN,[1] it is possible to produce hæmoglobin from hæmochromogen and globin, and it is indeed possible that other proteins can replace globin in this formation.

Hæmatoidin, thus called by VIRCHOW, is a pigment which crystallizes in orange-colored rhombic plates, and which occurs in old blood extravasations, and whose origin from the blood-coloring matters seems to be established (LANGHANS, CORDUA, QUINCKE, and others[2]). A solution of hæmatoidin shows no absorption-bands, but only a strong absorption from the violet to the green (EWALD[3]). According to most observers, hæmatoidin is identical with the bile-pigment bilirubin. It is not identical with the crystallizable lutein from the *corpora lutea* of the ovaries of the cow (PICCOLO and LIEBEN,[4] KÜHNE and EWALD).

In the detection of the above-described blood-coloring matters the spectroscope is the only entirely trustworthy means of investigation. If it is only necessary to test for blood in general and not to determine definitely whether the coloring-matter is hæmoglobin, methæmoglobin or hæmatin, then the preparation of hæmin crystals is an absolutely positive test. In regard to the detection of blood in urine, see Chapter XIV, and for the detection of blood in intestinal contents, in pathological fluids and in chemico-legal cases we must refer the reader to more extended text-books.

The methods proposed for the quantitative estimation of the blood-coloring matters are partly chemical and partly physical.

Among the chemical methods to be mentioned is the incineration of the blood and the determination of the amount of iron contained in the ash from which the amount of hæmoglobin may be calculated. We must refer to works on chemical methods of investigation in regard to these methods.

The physical methods consist either of colorimetric or of spectroscopic investigations.

The principle of HOPPE-SEYLER'S *colorimetric method* is that a measured quantity of blood is diluted with an exactly measured quantity of water until the diluted blood solution has the same color as a pure oxyhæmoglobin solution of a known strength. The amount of coloring-matter present in the undiluted blood may be easily calculated from the degree of dilution. In the colorimetric testing we use a glass vessel with parallel

[1] Zaleski, Zeitschr. f. physiol. Chem., 43; Laidlaw, Journ. of Physiol., 31; Ham and Balean, *ibid.*, 32.

[2] A comprehensive review of the literature pertaining to hæmatoidin may be found in Stadelmann, Der Icterus, etc., Stuttgart, 1891, pp. 3 and 45.

[3] Zeitschr. f. Biologie, 22, 475.

[4] Cit. from Gorup-Besanez, Lehrbuch d. physiol. Chem., 4. Aufl., 1878.

sides containing a layer of liquid 1 cm. thick (HOPPE-SEYLER's hæmatinometer). The use of HOPPE-SEYLER's colorimetric double pipette is more advantageous. Other good forms of apparatus have been constructed by GIACOSA and ZANGERMEISTER.[1] Instead of an oxyhæmoglobin solution we now generally use a carbon-monoxide hæmoglobin solution as a standard liquid because it may be kept for a long time. The blood solution in this case is saturated with carbon monoxide.[2]

The quantitative estimation of the blood-coloring matters by means of the spectroscope may be done in different ways, but at the present time the *spectrophotometric* method is chiefly used, and this seems to be the most reliable. This method is based on the fact that the extinction coefficient of a colored liquid for a certain region of the spectrum is directly proportional to the concentration, so that $C : E = C_1 : E_1$, when C and C_1 represent the different concentrations and E and E_1 the corresponding coefficients of extinction. From the equation $\frac{C}{E} = \frac{C_1}{E_1'}$ it follows that for one and the same pigment this relation, which is called the *absorption ratio*, must be constant. If the absorption ratio is represented by A, the determined extinction coefficient by E, and the concentration (the amount of coloring-matter in grams in 1 cc.) by C, then $C = AE$.

Different forms of apparatus have been constructed (VIERORDT and HÜFNER[3]) for the determination of the extinction coefficient, which is equal to the negative logarithm of those rays of light which remain after the passage of the light through a layer 1 cm. thick of an absorbing liquid. In regard to this apparatus the reader is referred to other textbooks.

For purposes of control the extinction coefficients are determined in two different regions of the spectrum. HÜFNER has selected (*a*) the region between the two absorption-bands of oxyhæmobglobin, especially between the wave-lengths 554 $\mu\mu$ and 565 $\mu\mu$ and (*b*) the region of the second band, especially the interval between the wave-lengths 531.5 $\mu\mu$ and 542.5 $\mu\mu$. The constants or the absorption ratio for these two regions of the spectrum are designated by HÜFNER by A and A'. Before the determination the blood must be diluted with water, and if the proportion of dilution of the blood be represented by V, then the concentration or the amount of coloring-matter in 100 parts of the undiluted blood is

$$C = 100.\ V.\ A.\ E \text{ and}$$
$$C = 100.\ V.\ A'.\ E'.$$

The absorption ratio or the constants in the two above-mentioned regions of the spectrum have been determined for oxyhæmoglobin, hæmoglobin, carbon-monoxide hæmoglobin, and methæmoglobin, as follows:

Oxyhæmoglobin	A_o	$=0.002070$ and $A'_o = 0.001312$
Hæmoglobin	A_r	$=0.001354$ and $A'_r = 0.001778$
Carbon-monoxide hæmoglobin	A_c	$=0.001383$ and $A'_c = 0.001263$
Methæmoglobin	A_m	$=0.002077$ and $A'_m = 0.001754$

[1] F. Hoppe-Seyler, Zeitschr. f. physiol. Chem., **16**; G. Hoppe-Seyler, *ibid.*, **21**; Winternitz, *ibid.*; Giacosa, Maly's Jahresber., **26**; Zangermeister, Zeitschr. f. Biologie, **33**.

[2] See Haldane, Journ. of Physiol., **26**.

[3] See Vierordt. Die Anwendung des Spektralapparates zu Photometrie, etc. (Tübingen, 1873), and Hüfner. Arch. f. (Anat. u.) Physiol., 1894, and Zeitschr. f. physiol Chem., **3**; v. Noorden, *ibid.*, **4**; Otto, Pflüger's Arch, **31** and **36**.

From what has been said above about the absorption behavior, the concentration and the extinction coefficient it follows that the quotient of the extinction coefficient $\frac{E'}{E}$ measured at two different parts of the spectrum, independently of the concentration, is a characteristic constant for the respective pigments. According to HÜFNER'S figures this quotient for oxyhæmoglobin is 1.58, for hæmoglobin 0.76, for carbon-monoxide hæmoglobin 1.10 and for methæmoglobin 1.19. BUTTERFIELD[1] who has made a thorough investigation on this, finds the figure 1.58 for normal and pathological human blood as well as for crystalline human, horse and ox oxyhæmoglobin.

The quantity of each coloring-matter may be determined in a mixture of two blood-coloring matters by this method; this is of special importance in the determination of the quantity of oxyhæmoglobin and hæmoglobin present in blood at the same time.

In order to facilitate these determinations, HÜFNER[2] has worked out tables which give the relation between the two pigments existing in a solution containing oxyhæmoglobin and another pigment (hæmoglobin, methæmoglobin, or carbon-monoxide hæmoglobin), and thus allowing of the calculation of the absolute quantity of each pigment.

Among the many apparati constructed for clinical purposes for the quantitative estimation of hæmoglobin, FLEISCHL'S *hæmometer*, which has undergone numerous modifications, HÉNOCQUE'S *hæmatoscope*, and SAHLI'S *hæmometer*, are to be specially mentioned. In regard to these apparati we must refer to larger hand-books and text-books on clinical methods.

Many other pigments are found besides the often-occurring hæmoglobin in the blood of invertebrates. In a few Arachnidæ, Crustacea, Gasteropodæ and Cephalopodæ a body analogous to hæmoglobin, containing copper, *hæmocyanin*, has been found by FREDERICQ. By the taking up of loosely bound oxygen this body is converted into blue *oxyhæmocyanin*, and by the escape of the oxygen becomes colorless again. According to HENZE 1 gram hæmocyanin combines with about 0.4 cc. oxygen. It is crystalline and has the following composition: C 53.66; H 7.33; N 16.09; S 0.86; Cu 0.38; O 21.67 per cent. On hydrolytic cleavage with hydrochloric acid HENZE found the following division of the nitrogen in hæmocyanin: Of the total nitrogen 5.78 per cent was split off as ammonia, 2.67 per cent as humus nitrogen, 27.65 per cent as diamino nitrogen, and 63.39 per cent as monamino nitrogen. He found no arginine in the cleavage products, but could detect histidine, lysine, tyrosine, and glutamic acid. A coloring-matter called *chlorocruorin* by LANKESTER is found in certain Chætopodæ. *Hæmerythrin*, so called by KRUKENBERG but first observed by SCHWALBE, is a red coloring-matter from certain Gephyrea. Besides hæmocyanin we find in the blood of certain Crustacea the red coloring-matter *tetronerythrin* (HALLIBURTON), which is also widely spread in the animal kingdom. *Echinochrom*, so named

[1] Zetischr. f. physiol. Chem., 62.
[2] Arch. f. (Anat. u.) Physiol., 1900.

by MacMunn,[1] is a brown coloring-matter occurring in the perivisceral fluid of a variety of echinoderms. According to Henze [2] the Ascidia contain a brown pigment which contains vanadium but does not hold any oxygen in a dissociable form.

The *quantitative constitution of the red blood-corpuscles.* The amount of water varies in different varieties of blood-corpuscles between 570–644 p. m., with a corresponding amount, 430–356 p. m., of solids. The chief mass, about $\frac{8}{10}$–$\frac{9}{10}$, of the dried substance consists of hæmoglobin (in human and mammalian blood).

According to the analyses of Hoppe-Seyler [3] and his pupils, the red corpuscles contain in 1000 parts of the dried substance:

	Hæmoglobin.	Protein.	Lecithin.	Cholesterin.
Human blood.....	868–944	122–51	7.2–3.5	2.5
Dog's "	865	126	5.9	3.6
Goose's "	627	364	4.6	4.8
Snake's "	467	525	...	...

Abderhalden found the following composition for the blood-corpuscles from the domestic animals investigated by him: Water, 591.9–644.3 p. m.; solids 408.1–335.7 p. m.; hæmoglobin, 303.3–331.9 p. m.; protein, 5.32 (dog)–7.85 p. m. (sheep); cholesterin, 0.388 (horse) –3.593 p. m. (sheep); and lecithin, 2.296 (dog)–4.855 p. m.

Of special interest is the varying proportion of the hæmoglobin to the protein in the nucleated and in the non-nucleated blood-corpuscles. These last are much richer in hæmoglobin and poorer in protein than the former.

The amount of mineral bodies in various species of animals is different. According to Bunge and Abderhalden the red corpuslces from the pig, horse, and rabbit contain no soda, while those from man, the ox, sheep, goat, dog, and cat are relatively rich in soda. In the five last-mentioned species the amount of soda was 2.135–2.856 p. m. The quantity of potash was 0.257 (dog)–0.744 p. m. (sheep). In the horse, pig, and rabbit the quantity of potash was 3.326 (horse)–5.229 p. m. (rabbit). Human blood-corpuscles contain, according to Wanach, about five times as much potash as soda, on an average 3.99 p. m. potash and 0.75 p. m. soda. The nucleated erythrocytes of the frog, toad, and turtle also

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[1] Fredericq, Extrait des Bulletins de l'Acad. Roy. de Belgique (2), **46**, 1878; Lankester, Journ. of Anat. and Physiol., **2** and **4**; Henze, Zeitschr. f. physiol. Chem., **33** and **43**; Krukenberg, see Vergl. physiol. Studien Reihe 1, Abt. 3, Heidelberg, 1880; Halliburton, Journal of Physiol., **6**; MacMunn. Quart. Journ. Microsc. Science, 1885.

[2] Zeitschr. f. physiol. Chem., **72** and **79**.

[3] Med.-chem. Untersuch., 390 and 393.

contain, according to BOTTAZZI and CAPPELLI,[1] considerably more potassium than sodium. Lime is claimed to be absent in the blood-corpuscles, but according to HAMBURGER[2] this is not true for at least ox-blood, and magnesia occurs only in small amounts: 0.016 (sheep) –0.150 p. m. (pig). The blood-corpuscles of all animals investigated contain chlorine, 0.460–1.949 p. m. (both in horse), generally 1 to 2 p. m., and also phosphoric acid. The amount of inorganic phosphoric acid shows great variation: 0.275 (sheep)–1.916 p. m. (horse). All of the above figures are calculated on the fresh, moist blood-corpuscles.

By quantitative determinations of the swelling and shrinking of the cells under the influence of NaCl solutions of various concentration, or of serum of various dilutions, HAMBURGER has attempted to determine for the erythrocytes, as well as the leucocytes, the percentage relationship between the two chief constituents of the cells (the frame and the intracellular fluid). He found that the volume of the frame-substance for both varieties of blood-corpuscles of the horse was equal to 53–56.1 per cent. The volume for the red blood-corpuscles was for the rabbit 48.7–51; hen, 52.4–57.7, and for the frog, 72–76.4 per cent. KOEPPE has raised objections to these determinations.[3]

The White Blood-corpuscles and the Blood-plates.

The White Blood-corpuscles, also called LEUCOCYTES or Lymphoid Cells, are of different kinds, and ordinarily we differentiate between the small forms poor in protoplasm, called lymphocytes, and the larger, granular, often more nucleated forms, called leucocytes. The polynuclear leucocytes occur in greater abundance in the blood than the lymphocytes. In human and mammalian blood, most of the white blood-corpuscles are larger than the red blood-corpuscles. They also have a lower specific gravity than the red corpuscles, move in the circulating blood nearer to the walls of the blood-vessels, and also have a slower motion.

The number of white blood-corpuscles varies not only in the different blood-vessels, but also under different physiological conditions. On an average there is only 1 white corpuscle for 350–500 red corpuscles. According to the investigations of ALEX. SCHMIDT[4] and his pupils, the leucocytes are destroyed in great part on the discharge of the blood before and during coagulation, so that discharged blood is much poorer in leucocytes than the circulating blood. The correctness of this statement has been denied by other investigators.

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[1] Bunge, Zeitschr. f. Biologie, **12**, and Abderhalden, Zeitschr. f. physiol. Chem., **23** and **25**; Wanach, Maly's Jahresber., 18, 88; Bottazzi and Cappelli, Arch. Ital. de Biologie, **32**.

[2] Zeitschr. f. physik. Chem. **69**.

[3] Hamburger, Arch. f. (Anat. u ' Physiol., 1898; Koeppe, *ibid.*, 1899 and 1900.

[4] Pflüger's Arch., 11 and Krüger, Arch. f. exp. Path. u. Pharm., **51**.

From a histological standpoint we generally, as above indicated, discriminate between the different kinds of colorless blood-corpuscles. Chemically considered, however, there is no known essential difference between them, and what little we do know chemically is chiefly in connection with the leucocytes. With regard to their importance in the coagulation of fibrin, ALEX. SCHMIDT and his pupils distinguish between the leucocytes which are destroyed in the coagulation and those which are not. The last mentioned give with alkalies or common-salt solutions a slimy mass; the first do not show such behavior.

The protoplasm of the leucocytes has, during life, amœboid movements which serve partly to make possible the wandering of the cells, and partly to aid in the absorption of smaller grains or foreign bodies and make the phagocytosis possible. The action of various agents such as hyper- and hypotonic salt solutions, of foreign ions, such as iodine, bromine, and salts of the alkaline earths upon the chemotaxis and the phagocytic activity of the leucocytes has been thoroughly studied by HAMBURGER and DE HAAN,[1] and among other things they have shown that the Ca causes an accellerating influence upon phagocytosis which is peculiar for Ca and does not depend upon its properties as a divalent ion. Because of the contractibility of the leucocytes, the occurrence of *myosin* in them has been admitted even without any special proof therefore. We know nothing positively whether in the leucocytes, or in the cells, in general, globulins occur with traces of albumins, because cell constituents which used to be called globulins have on more careful investigation been found to be nucleoalbumins or nucleoproteins. The substance observed by HALLIBURTON,[2] and occurring in all cells, which coagulates at 47 to 50° C., is considered as a true globulin. ALEX. SCHMIDT claims to have found serglobulin in equine-blood leucocytes which have been washed with ice-cold water.

The proteins of the leucocytes as well as the cells in general are principally compound proteins. For the present it is impossible to state to what extent the nucleoalbumins occur in leucocytes or cells, because in the past no careful differentiation was made between the nucleoalbumins and nucleoproteins. The nucleoproteins are without any doubt the principal constituents of the protoplasm of the white blood-corpuscles, and one of these it seems is identical with the so-called hyaline substance of ROVIDA, which yields a slimy mass when treated with alkalies or NaCl solutions and which occur in pus-cells.

On digesting the leucocytes with water, a solution of a protein body

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[1] Bioch. Zeitschr., **24** and **26**.

[2] See Halliburton, On the chem. Physiol. of the animal cell. King's College, London, Physiol. Labor. Collected papers, 1893.

is obtained which can be precipitated by acetic acid and which forms the chief mass of the leucocytes. This substance, which is undoubtedly concerned in the coagulation of the blood, has been described under different names, such as *tissue fibrinogen* (WOOLDRIDGE) *cytoglobin* and *präglobulin* (ALEX. SCHMIDT) or *nucleohistone* (KOSSEL and LILIENFELD [1]) and consists, chiefly at least, of nucleoprotein. The ordinary view that this is nucleohistone does not seem to be correct, according to the investigations of BANG,[2] and further proof is necessary.

Besides these constituents of the protoplasm of the leucocytes we must also include *lecithin* and especially *phosphatides*, *cholesterin*, *glucothionic acid* (in pus-corpuscles, MANDEL and LEVENE [3]), *purine bodies* derived from the nuclein substances and *glycogen*. According to HOPPE-SEYLER glycogen is a constant constituent of all cells having amœboid movement, and he found it in the colorless blood-corpuscles but not in the non-mobile pus-cells. Nevertheless glycogen has also been found in pus-cells by SALOMON [4] and by others. The glycogen found by HUPPERT, CZERNY, DASTRE,[5] and others in blood and lymph probably originated from the leucocytes. *Enzymes* also occur in the leucocytes and the proteolytic enzymes are of special importance. According to OPIE and BARKER two proteolytic enzymes occur in the leucocytes, one of which is active in alkaline solution and occurs in the polynuclear cells while the other is active in acid solution and occurs in the large mononuclear cells. According to FIESSINGER and MARIE, the leucocytes contain a proteolytic enzyme which forms peptone, leucine and tyrosine from protein and which is probably identical with the proteolytic enzyme discovered earlier by ACHALME in pus. It acts best in faintly alkaline solution, but also in weak acid reaction, and is destroyed at 75–80° C. It occurs in the polynuclear leucocytes but principally in those which have a medullary origin, while it is absent in the leucocytes of the lymph series. The *lipase* occurring in pus and in blood seems, according to the above experimenters, to originate in the lymphocytes. TSCHERNORUZKI [6] has

[1] See Wooldridge, Die Gerinnung des Blutes (published by M. v. Frey, Leipzig, 1891); A. Schmidt, Zur Blutlehre, Leipzig, 1892; Lilienfeld, Zeitschr. f. physiol. Chem., 18.

[2] I. Bang, Studier over Nukleoproteider, Kristiania, 1902.

[3] Biochem. Zeitschr., 4.

[4] In regard to the literature on Glycogen, see Chapter VII.

[5] Huppert, Centralbl. f. Physiol., 6, 394; Czerny, Arch. f. exp. Path. u. Pharm., 31; Dastre, Compt. Rend., 120, and Arch. de Physiol. (5), 7. See also Hirschberg, Zeitschr. f. klin. Med., 54.

[6] In regard to the enzymes see Erben, Jochmann and E. Müller, Jochmann and Lockemann, Hofmeister's Beiträge, 11, which contains the literature. Opie, Journ. of exper. Medicine, 8; with Barker, *ibid.*, 9; Fiessinger, and Marie, Journ. de physiol. et de pathol. générale, 11, which also contains the literature and Compt. rend. soc. biol., 66, 67; Tschernoruzki, Zeitschr. f. physiol. Chem., 75.

also shown the presence of *amylase* (diastase), *catalase*, *nuclease* and *peroxidase* in the polynuclear leucocytes.

The **blood-plates** (BIZZOZERO), hæmatoblasts (HAYEM), whose nature, preformed occurrence, and physiological importance have been much questioned, are pale, colorless, gummy disks, round or somewhat oval in shape, generally with a diameter one-half or one-third that of the blood-corpuscles. In mammalia their number, according to AYNAUD, is on an average 500,000 in 1 c.mm. They change their shape readily, attack foreign bodies and agglutinate under conditions which AYNAUD has carefully studied. Human blood-plates consist, according to DEETJEN,[1] of a nucleus and a hyaline protoplasm. They are very sensitive toward alkalies and much more so than the plates from other mammalia. They are destroyed in a concentration of hydroxyl ions, $C_{OH}=1\times10^{-5}$ and in a concentration of H ions, $C_H=2\times10^{-4}$.

According to the researches of KOSSEL and of LILIENFELD [2] the blood-plates consist of a chemical combination between protein and nuclein, and hence they are also called *nuclein-plates* by LILIENFELD, and are considered as derivatives of the cell nucleus. It seems certain that the blood-plates have some connection with the coagulation of blood. The views on this question, especially in regard to the manner in which these plates act in coagulation, are unfortunately very divergent.

III. THE BLOOD AS A MIXTURE OF PLASMA AND BLOOD-CORPUSCLES.

The blood in itself is a thick, sticky, light or dark red liquid, opaque even in thin layers, having a salty taste and a faint odor differing in different kinds of animals. On the addition of sulphuric acid to the blood the odor is more pronounced. In adult human beings the specific gravity ranges between 1.045 and 1.075. It has an average of 1.058 for grown men and a little less for women. LLOYD JONES found that the specific gravity is highest at birth and lowest in children until about two years old, and in pregnant women. The determinations of LLOYD JONES, HAMMERSCHLAG,[3] and others show that the variation of the specific gravity, dependent upon age and sex, corresponds to the variation in the quantity of hæmoglobin.

The determination of the specific gravity is accurately obtained

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[1] Aynaud, Maly's Jahresb., **29**; Deetjen, Zeitschr. f. physiol. Chem., **68**.

[2] In regard to the literature of the blood-plates, see Lilienfeld, Arch. f. (Anat. u.) Physiol., 1892, and "Leukocyten und Blutgerrinnung," Verhandl. d. physiol. Gesellsch. zu Berlin, 1892; and also Mosen, Arch. f. (Anat. u.) Physiol., 1893, and Maly's Jahresber., **30** and **31**.

[3] Lloyd Jones, Journ. of Physiol., 8; Hammerschlag, Wien. klin. Wochenschrift, 1890, and Zeitschr. f. klin. Med., **20**.

by means of the pyknometer. For clinical purposes, where only small amounts are available, it is best to proceed by the method as suggested by HAMMERSCHLAG. Prepare a mixture of chloroform and benzene of about 1.050 sp. gr. and add a drop of the blood to this mixture. If the drop rises to the surface then add benzene, and if it sinks add chloroform. Continue this until the drop of blood suspends itself midway and then determine the specific gravity of the mixture by means of an areometer. This method is not strictly accurate and must be performed quickly. In regard to the necessary details refer to ZUNTZ and A. LEVY.[1]

The reaction of the blood is alkaline toward litmus, and various bodies such as alkali carbonates, the phosphates, alkali-protein combinations, the amino-acids and carbon dioxide all take part in bringing about the normal reaction. According to HENDERSON[2] the normal reaction is also partly brought about by ammonia formation and partly by the phosphates, in that the kidneys secrete acid salts (phosphates) and return alkali to the blood and regulate the reaction of the blood.

In considering the alkalinity of the blood we must, as previously remarked, differentiate between the amount of titratable alkali in the blood and the true alkalinity, i.e., the amount of hydroxyl or hydrogen ions in the blood.

We have a large number of determinations of the quantity of titratable alkali, calculated as Na_2CO_3, in fresh as well as defibrinated blood of animals and man, and in the latter case under healthy and diseased conditions. As these determinations have been carried out with different methods which were not without error they cannot be given any great importance. The results found generally vary between 3 and 6 p. m. Na_2CO_3 and for man the figures below 3.3 p. m. and above 5.3 p. m. are considered as pathological. The alkaline reaction diminishes outside of the body, and indeed the more quickly the greater the original alkalinity of the blood. This depends on the formation of acid in the blood, in which the red-blood corpuscles seem to take part in some way or another. After excessive muscular activity the alkalinity is diminished (PEIPER, COHNSTEIN), and it is also decreased after the continuous ingestion of acids (LASSAR, FREUDBERG,[3]) and others.

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[1] Zuntz, Pflüger's Arch., **66**; Levy, Proceed. Roy. Soc., **71**.

[2] Amer. Journ. of Physiol., **21**, and Journ. of biol. Chem., **9**; see also Robertson, *ibid.*, **6** and **7**.

[3] Peiper, Virchow's Arch., **116**; Cohnstein, *ibid.*, **130**, which also cites the works of Minkowski, Zuntz, and Geppert; Freudberg, *ibid.*, **125** (literature); in regard to the methods for the estimation of the alkalinity see, besides the above-mentioned authors, v. Jaksch, Klin. Diagnostik; v. Limbeck, Wien. med. Blätter, **18**; Wright, The Lancet, **1897**; Biernacki, Beiträge zur Pneumatologie, etc., Zeitschr. f. klin. Med., **31** and **32**; Hamburger, Eine Methode zur Trennung, etc., Arch., f. (Anat. u.) Physiol.,

The methods for the determination of the true reaction of animal fluids, also the blood, have been given in Chapter I. For the true alkalinity of the blood, as first shown by HÖBER and especially by HASSELBALCH and LUNDSGAARD, the carbon dioxide is of the greatest importance in that with an increasing carbon-dioxide tension the concentration of the H ions increase. Thus HASSELBALCH and LUNDSGAARD [1] found that a rise in the carbon-dioxide tension of 30–50 mm., that is a rise of 20 mm., increased the concentration of the H ions about 36 per cent.

For the determination of the true reaction the temperature at which the measurement is made is of the greatest importance. As the dissociation constant of water strongly rises with the temperature, the HO ion concentration of the blood must rise with the temperature, and we can believe that the alkalinity of the blood at body temperature must be 2–3 times greater than when measured at 18° and that this alkalinity increases 15–20 per cent when the normal temperature of the body (38°) rises to that of a high fever (42°).

The true alkalinity of the blood is somewhat variable under different conditions. In this connection it must be remarked that also age and other conditions have an action upon the alkalinity. As the determinations are made with different, and not always exact methods, and sometimes without consideration of the action of carbon dioxide and temperature, it is extremely difficult to give satisfactory average results. Under these circumstances it is perhaps sufficient to refer to the figures given in Chapter I (page 76).

The alkali of the blood as above mentioned exists in part as alkaline salts, carbonate and phosphate, and partly in combination with protein or hæmoglobin. The first are often spoken of as readily diffusible alkalies, while the others are not or are only diffusible with difficulty (see page 268). The quantity of the first, in human blood, is about one-fifth of the total alkali (BRANDENBURG). The readily as well as the difficultly diffusible alkali is divided between the blood-corpuscles and plasma, and the blood-corpuscles seem to be richer in difficultly diffusible alkali than the plasma or serum. This division may be changed by the influence of even very small amounts of acid, even of carbonic acid, and also, as shown by ZUNTZ, LOEWY and ZUNTZ, HAMBURGER, LIMBECK, and GÜRBER,[2]

1898. See also Maly's Jahresber., **29**, **30**, and **31**; Salaskin and Pupkin, Zeitschr. f. physiol. Chem., **42**, and O. Folin, *ibid.*, **43**; Laitinen, Hammarsten's Festschr., 1903; Westenrijk, Arch. f. exp. Path. u. Pharm. Suppl., 1908, Schmiedeberg-Festschrift.

[1] The literature may be found in Sörenssen, Messung und Bedeutung der Wasserstoff-ionkonzentrationen, Ergbn. d. Physiol., **12**; Hasselbalch and Lundsgaard, Bioch. Zeitschr., **38**, and Skand. Arch. f. Physiol., **27**; Hasselbalch, Bioch. Zeitschr., **30**; Lundsgaard, *ibid.*, **41**.

[2] Zuntz, in Hermann's Handbuch der Physiol., **4**, Abt. 2; Loewy and Zuntz,

by the influence of the respiratory exchange of gas. The blood-corpuscles give up a part of the alkali united with protein to the serum by the action of carbon dioxide, hence the serum becomes more alkaline. The equilibrium of the osmotic tension in the blood-corpuscles and in the serum is thus disturbed; the blood-corpuscles swell up because they take up water from the serum, and this then becomes more concentrated and richer in alkali, protein, and sugar. Under the influence of oxygen, the corpuscles take their original form again and the above changes are reversed. The blood-corpuscles for this reason are less biconcave in their small diameter in venous than in arterial blood (HAMBURGER).

These conditions have been further studied by v. KORÁNYI and BENCE,[1] and they have investigated the relation between the changes of the volume of the blood-corpuscles and the electrical conductivity, the refractivity of the serum and the viscosity of the blood. The refraction coefficient of the serum is highest with an increase in the amount of carbon dioxide, while it is lowest when the blood is rich in oxygen and poor in carbon dioxide. They consider this as an action of acid, as a similar rise is observed after the addition of acid, while after the addition of alkali a fall in the refraction coefficient of the serum takes place, and these same changes can be brought about by CO_2 or by a current of oxygen. With an increase in the amount of carbon dioxide, the conductivity of the blood diminishes; the viscosity is, on the other hand, highest when the blood is richest in carbon dioxide. If the CO_2 is driven off by O the viscosity diminishes to a minimum, and on leading in more oxygen it rises again. The changes in viscosity of the blood runs parallel with the volume changes of the blood-corpuscles, and changes in the viscosity, which can be brought about by the removal of carbon dioxide, cause a change in the electric charge of the blood-corpuscles (v. KORÁNYI and BENCE). The viscosity of the blood is a variable quantity which, besides the gas content of the blood, is also dependent upon many other circumstances (ADAM[2]) and which is different at various ages and under unequal physiological and pathological conditions.

The color of the blood is red—light scarlet-red in the arteries and dark bluish-red in the veins. Blood free from oxygen is dichroic, dark red by reflected light and green by transmitted light. The blood-coloring matters occur in the blood-corpuscles. For this reason blood is opaque

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Pflüger's Arch., 58; Hamburger, Arch. f. (Anat. u.) Physiol., 1894 and 1898, and Zeitschr. f. Biologie, 28 and 35; v. Limbeck, Arch. f. exp. Path. u. Pharm., 35; Gürber, Sitzungsber. d. phys. med. Gesellsch. zu Würzburg, 1895.

[1] Pflüger's Arch., 110.

[2] In regard to the viscosity of the blood and the literature of the subject, see R. Höber in Oppenheimer's Handb. der Bioch., 2, p. 12–18. See also Adam, Zeitschr. f. klin. Med., 68.

in thin layers. If the hæmoglobin is removed from the stroma and dissolved by the blood liquid by any of the above-mentioned means (see page 273), the blood becomes transparent and has then a "*lake color*."[1] Less light is now reflected from its interior, and this laky blood is therefore darker in thicker layers. On the addition of salt solutions to the blood-corpuscles they shrink, more light is reflected, and the color appears lighter. A great abundance of red corpuscles makes the blood darker, while by diluting with serum or by a greater abundance of white corpuscles the blood becomes lighter in appearance. The different colors of arterial and of venous blood depend on the varying quantities of gas contained in these two varieties of blood, or, better, on the different amounts of oxyhæmoglobin and hæmoglobin they contain.

The most striking property of blood consists in its coagulating within a shorter or longer time, but as a rule very shortly after leaving the veins. Different kinds of blood coagulate with varying rapidity; in human blood the first marked sign of coagulation is seen in two to three minutes, and within seven to eight minutes the blood is thoroughly converted into a gelatinous mass. If the blood is allowed to coagulate slowly, the red corpuscles have time to settle more or less before the coagulation, and the blood-clot then shows an upper yellowish-gray or reddish-gray layer consisting of fibrin enclosing chiefly colorless corpuscles. This layer has been called *crusta inflammatoria* or *phlogistica*, because it has been especially observed in inflammatory processes and is considered one of the characteristics of them. This crusta, or "*buffy coat*," is not characteristic of any special disease, and it occurs chiefly when the blood coagulates slowly or when the blood-corpuscles settle more quickly than usual. A buffy coat is often observed in the slowly coagulating equine blood. The blood from the capillaries is not supposed to have the power of coagulating.

Coagulation is retarded by cooling, by diminishing the oxygen, and by increasing the amount of carbon dioxide, which is the reason that venous blood and to a much higher degree blood after asphyxiation coagulates more slowly than arterial blood. The coagulation may be retarded or prevented by the addition of acids, alkalies, or ammonia, even in small quantities; by concentrated solutions of neutral alkali salts and alkaline earths, alkali oxalates and fluorides; also by egg-albumin, solutions of sugar or gum, glycerin, or much water; also by receiving the blood in oil. Coagulation may be prevented by the injection of a proteose solution or of an infusion of the leech into the circulating blood, but this

[1] R. Du Bois-Reymond presents objections to the general use of the above terms in Centralbl. f. Physiol., 19, p. 65.

infusion also acts in the same way on blood just drawn. Coagulation is also hindered by snake poison (cobra-poison), and bacterial toxines. The coagulation may be facilitated by raising the temperature; by contact with foreign bodies, to which the blood adheres; by stirring or beating it; by admission of air; by diluting with very small amounts of water; by the addition of platinum-black or finely powdered carbon; by the addition of laky blood, which does not act by the presence of dissolved blood-coloring matters, but by the stromata of the blood-corpuscles; and also by the addition of the leucocytes from the lymphatic glands, or of a watery saline extract of the lymphatic glands, testicles, or thymus and various other organs (DELEZENNE, WRIGHT, ARTHUS,[1] and others).

An important question to answer is why the blood remains fluid in the circulation, while it quickly coagulates when it leaves the circulation. The reason why blood coagulates on leaving the body is therefore to be sought for in the influence which the walls of the living and uninjured blood-vessels exert upon it. These views are derived from the observations of many investigators. From the observations of HEWSON, LISTER, and FREDERICQ it is known that when a vein full of blood is ligatured at the two ends and removed from the body, the blood may remain fluid for a long time. BRÜCKE[2] allowed the heart removed from a tortoise to beat at 0° C., and found that the blood remained uncoagulated for some days. The blood from another heart quickly coagulated when collected over mercury. In a dead heart, as also in a dead blood-vessel, the blood soon coagulates, and also when the walls of the vessel are changed by pathological processes.

What then is the influence which the walls of the vessels exert on the liquidity of the circulating blood? FREUND found that the blood remains fluid when collected by means of a greased canula under oil or in a vessel smeared with vaseline. If the blood collected in a greased vessel be beaten with a glass rod previously oiled, it does not coagulate, but it quickly coagulates on beating it with an unoiled glass rod or when it is poured into a vessel not greased. The non-coagulability of blood collected under oil was confirmed later by HAYCRAFT and CARLIER. FREUND found on further investigation that the evaporation of the upper layers of blood or their contamination with small quantities of dust causes a coagulation even in a vessel treated with vaseline. According

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[1] Delezenne, Arch. de Physiol. (5), 8; Wright, Journ. of Physiol., 28; Arthus, Journ. de Physiol. et Pathol., 4.

[2] Hewson's works, edited by Gulliver, London, 1876, cited from Gamgee, Textbook of Physiol. Chem., 1, 1880; Lister, cited from Gamgee, *ibid.*; Fredericq, Recherches sur la constitution du plasma sanguin, Gand, 1878; Brücke, Virchow's Arch. 12.

to FREUND [1] it is this adhesion between the blood and a foreign substance —and the diseased walls of the vessel also act as as such—that gives the impulse toward coagulation, while the lack of adhesion prevents the blood from coagulating. BORDET and GENGOU [2] have also shown that the plasma obtained by centrifuging blood collected in a paraffined vessel, and perfectly free from form-elements, can be kept without coagulating in a paraffined vessel, and that it does coagulate on being transferred to an unparaffined vessel. The adhesion of the plasma to a foreign body may also, in the absence of form-elements, give the impulse to coagulation. That this adhesion of the form-elements is of great importance cannot be denied and is also generally accepted. By this adhesion the form-elements undergo certain changes which seem to stand in a certain relation to the coagulation of the blood.

The views in regard to these changes are, unfortunately, very divergent. According to ALEX. SCHMIDT [3] and the Dorpat school an abundant destruction of the leucocytes, especially polynuclear leucocytes, takes place in coagulation, and important constituents for the coagulation of the fibrin pass into the plasma. A direct relation between the destruction of leucocytes and coagulation is denied by many investigators, while according to other experimenters the essential factor is not a destruction of the leucocytes, but an elimination of constituents from the cells into the plasma. This process is called *plasmoschisis* by LÖWIT.[4] The passage of cell constituents into the plasma before coagulation must not necessarily be considered as a phenomenon of death, as it may just as well be a secretory process (ARTHUS, MORAWITZ, DASTRE [5]).

Great importance has also been ascribed to the blood-plates in coagulation as certain investigators (BIZZERO, LILIENFELD, SCHWALBE, MORAWITZ, BÜRKER, DEETJEN, LE SOURD and PAGNIEZ) found that they induce, accelerate or make coagulation possible. According to VINCI and CHISTONI they are not necessary as they are absent in the blood of birds, which coagulates rapidly, and also in the lymph, of the dog, rabbit and cat. They may nevertheless accelerate coagulation and they are necessary for the contraction of the clot. According to AYNAUD they

[1] Freund, Wien. med. Jahrb., 1886; Haycraft and Carlier, Journ. of Anat. and Physiol., **22**.

[2] Annal. de l'Institute Pasteur, **17**.

[3] Pflüger's Arch., **11**. The works of Alex. Schmidt are found in Arch. f. Anat. und Physiol., 1861, 1862; Pflüger's Arch., **6**, **9**, **11**, **13**. See especially Alex. Schmidt, Zur Blutlehre (Leipzig, 1892), which also gives the work of his pupils, and Weitere Beiträge zur Blutlehre, 1895.

[4] Wien. Sitzungsber., **89** and **90**, and Prager med. Wochenschr., 1889, referred to in Centralbl. f. d. med. Wissensch., 28, 265.

[5] Morawitz, Hofmeister's Beiträge, **5**; Arthus, Compt. rend. soc. biolog., **55**; Dastre, *ibid.*, **55**.

are not necessary for the contraction of the clot nor for the coagulation as a whole, and they are absent in the lymph and serous fluids. According to PETRONE [1] they indeed have a function in retarding coagulation.

WOOLDRIDGE [2] takes a very peculiar position in regard to this question: he considers the form-elements as only of secondary importance in coagulation. As he has found, a peptone-plasma which has been freed from all form-constituents by means of centrifugal force yields abundant fibrin when it is not separated from a substance which precipitates on cooling. This substance, which WOOLDRIDGE has called A-fibrinogen, seems to all appearances to be a nucleoproteid, which, according to the unanimous view of several investigators, originates from the form-elements of the blood, either the blood-plates or the leucocytes and the generally accepted view as to the great importance of the form-elements in the coagulation of the blood is not really contrary to WOOLDRIDGE'S experiments.

There is great diversity of opinion in regard to those bodies which are eliminated from the form-elements of the blood before and during coagulation.

According to ALEX. SCHMIDT the leucocytes, like all cells, contain two chief groups of constituents, one of which accelerates coagulation, while the other retards or hinders it. The first may be extracted from the cells by alcohol, while the other cannot be extracted. Blood-plasma contains only traces of thrombin, according to SCHMIDT, but does contain its antecedent, prothrombin. The bodies which accelerate coagulation are neither thrombin nor prothrombin, but they act in this wise in that they split off thrombin from the prothrombin. On this account they are called *zymoplastic substances* by ALEX. SCHMIDT. The nature of these bodies is unknown, and SCHMIDT has given no opinion as to their relation to the lime salts, which have been found to have zymoplastic activity by other investigators.

The constituents of the cells which hinder coagulation and which are insoluble in alcohol-ether are compound proteins, and have been called *cytoglobin* and *preglobulin* by SCHMIDT. The retarding action of these bodies may be suppressed by the addition of zymoplastic substances, and the yield of fibrin on coagulation in this case is much greater than in the absence of the compound protein retarding coagulation. This last supplies the material from which the fibrin is produced. The process is, according to SCHMIDT, as follows: The preglobulin first splits, yielding serglobulin, then from this the fibrinogen is derived, and from this latter the fibrin is produced. The object of the thrombin is two-fold. The thrombin first splits the fibrinogen from the paraglobulin, and then converts the

[1] See footnote 2, p. 308. Also Schwalbe, nters. z. Blutgerinnung, etc., Braunschweig, 1900; Morawitz, Deutsch. Arch. f. klin. Med., **79**, and Hofmeister's Beiträge, **4** and **5**; Bürker, Pflüger's Arch., **102**, and Centralbl. f. Physiol., **21**; Deetjen, l. c.; Le Sourd and Pagniez, Journ. de Physiol., **11**; Vinci and Chistoni, Chem. Centralbl., 1909, **2**, 838, and Maly's Jahresb., **39**; Aynaud, Maly's Jahresb., **39**; 165; Petrone, Maly's Jahresber., **31**, p. 170.

[2] Die Gerinnung des Blutes (published by M. v. Frey, Leipzig, 1891).

fibrinogen into fibrin. The assumption that fibrinogen can be split from paraglobulin has not sufficient foundation and is even improbable.

According to SCHMIDT the retarding action of the cells is prominent during life, while the accelerating action is especially pronounced outside of the body or by coming in contact with foreign bodies. The parenchymous masses of the organs and tissues, through which the blood flows in the capillaries, are those cell-masses which serve to keep the blood fluid during life.

LILIENFELD has given further proof as to the occurrence, in the form-elements of the blood, of bodies which accelerate or retard the coagulation. According to this author the nature of these bodies is very markedly different from SCHMIDT'S idea. While, according to SCHMIDT, the coagulation accelerators are bodies soluble in alcohol, and the compound proteins exhausted with alcohol act only retardingly on coagulation, LILIENFELD states that the substance which acts acceleratingly and retardingly on coagulation are contained in a nucleoprotein, namely, nucleohistone. Nucleohistone readily splits into leuconuclein and histone, the first of which acts as a coagulation-excitant, while the other, introduced into the blood-vascular system, either intravascular or extravascular, robs the blood of its property of coagulating. Introduced into the circulatory system the nucleohistone splits into its two components. It therefore causes extensive coagulation on one side and makes the remainder of the blood uncoagulable on the other. This theory as well as that of SCHMIDT is not based upon sufficiently demonstrated facts.

BRÜCKE showed long ago that fibrin left an ash containing calcium phosphate. The fact that calcium salts may facilitate or even cause a coagulation, in liquids poor in ferment, has been known for several years, through the researches of HAMMARSTEN, GREEN, RINGER and SAINSBURY. The necessity of the lime salts for the coagulation of blood and plasma was first shown positively by the important investigations of ARTHUS and PAGÈS. Recent investigations of SABBATANI [1] have also shown the importance of calcium salts or the free calcium ions for coagulation without explaining the mode of their action.

According to the generally accepted view of ARTHUS and PAGÈS the soluble lime salts precipitable by oxalate are necessary requisites for the fermentive transformation of fibrinogen, because thrombin remains inactive in the absence of soluble lime salts. This view is untenable, as shown by the researches of ALEX. SCHMIDT, PEKELHARING, and HAMMARSTEN.[2] Thrombin acts as well in the absence as in the presence of precipitable lime salts.

[1] Hammarsten, Nova Acta, reg. Soc. Scient. Upsala, (3), **10**, 1879; Green, Journ. of Physiol., 8; Ringer and Sainsbury, *ibid.*, 11 and 12; Arthus et Pagès and Arthus, see footnote 4, p. 251; Hammarsten, Zeitschr. f. physiol. Chem., **22**; Sabbatani, cited, Centralbl. f. Physiol., **16**, 665.

[2] Hammarsten, Zeitschr. f. physiol. Chem., **22**, where the other investigators are cited.

According to PEKELHARING[1] thrombin is the lime compound of prothrombin, and the process of coagulation consists, according to him, in the thrombin transferring the lime to the fibrinogen, which is thereby converted into an insoluble lime compound, fibrin. Among the objections to this theory can be mentioned, the fact that fibrin has not been obtained absolutely free from lime, but still so poor in lime (HAMMARSTEN[2]) that if the lime belongs to the fibrin, its molecule must be more than ten times greater than the hæmoglobin molecule, which is not probable. These as well as many other observations indicate that the lime is carried down by the fibrinogen only as a contamination.

If, as it seems, the lime is not of importance in the transformation of fibrinogen into fibrin in the presence of thrombin, still this does not contradict the above-mentioned observations of ARTHUS and PAGÈS that the lime salts are necessary for coagulation of blood and plasma. It is very probable that the lime salts, as admitted by PEKELHARING, are a requisite for the transformation of prothrombin into thrombin.

If we attempt to summarize the more or less contradictory investigations and views as given in the preceding pages, we can consider the following facts as conclusive: In the first place, two bodies, the fibrinogen and the thrombin, are necessary for the coagulation. The fibrinogen exists preformed in the plasma. The thrombin, on the contrary, does not occur in living blood, at least not in appreciable amounts as such, but is formed from another substance, the prothrombin. The presence of calcium salts is necessary for the formation of this thrombin, while the calcium salts are not necessary for the enzymotic transformation of fibrinogen into fibrin. Besides the calcium salts also other substances, the zymoplastic active substances, are active in the formation of thrombin from its mother-substance, and these zymoplastic substances stand in some relation to the form-elements of the blood.

The formation of thrombin and the relation of the form-elements therewith are still unexplained and disputed questions.

It is a question whether the mother-substance of thrombin exists in the plasma of the circulating blood or whether it is a body eliminated from the form-elements before coagulation. We have two opposing views on this question, namely, those of ALEX. SCHMIDT and of PEKELHARING. According to SCHMIDT prothrombin occurs preformed in the circulating plasma, and it is transformed into thrombin by the zymoplastic substances which pass out from the form-elements. PEKELHARING, on the contrary, holds the view that the plasma does not contain

[1] See footnote 4, p. 256, and especially Virchow's Festschrift, 1, 1891.
[2] Zeitschr. f. physiol Chem., 28.

appreciable amounts of prothrombin. This body, according to him, passes before coagulation from the form-elements into the plasma, and is there converted into thrombin by the calcium salts. The observation that uncoagulated leech-plasma does not coagulate on the addition of calcium salts, while it does coagulate on the addition of prothrombin solutions, seems to support this view; still it is not quite conclusive. Leech-extract contains a body, hirudin, which, seems to be an antibody toward thrombin and quantitatively neutralizes it. On the addition of prothrombin, new thrombin may be formed, which may act if the hirudin is not present in too great an excess.

Other observations which dispute the occurrence of prothrombin in the circulating plasma can be explained in various ways and it is the general view at present that the prothrombin is a preformed constituent of the plasma. [1]

Although the opinions are rather united as to the occurrence of at least three bodies, fibrinogen, prothrombin (thrombogen) and lime salts in the plasma, still the question arises how the thrombin is formed from the thrombogen. The zymoplastic substances must be here considered, and the starting-point in these new investigations is the accelerating action upon coagulation, of different tissue extracts, an action which has been known for a long time and was especially studied by DELEZENNE on the plasma from bird's blood. Unfortunately we are not in accord as to the nature and manner of action of the active constituents of these extracts. According to MORAWITZ the active body is not thrombin, but another substance called *thrombokinase*, besides lime-salts, which are necessary for the transformation of prothrombin (*thrombogen* according to MORAWITZ). The production of thrombokinase is, according to MORAWITZ, a general property of the protoplasm, and also occurs in the leucocytes (and blood-plates). Three substances are necessary, according to his view, for the formation of thrombin, namely: thrombogen, thrombokinase and lime salts. Thrombogen is, he claims, not quite identical with the prothrombin (other investigators), which he calls α-prothrombin, but is a mother-substance of it. The process of thrombin formation can be given as follows: The kinase first transforms the thrombogen into α-prothrombin, which latter then is converted into thrombin (α) by the lime salts.

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[1] Arthus, Journ. de Physiol. et Pathol., **3** and **4**, and Compt. rend. soc. biol., **56**. The works of Morawitz may be found in Hofmeister's Beiträge, **4** and **5**, Deutsch. Arch. f. klin. Med., **79** and **80**, and in Oppenheimer's Handb. der Bioch., **2**; Fuld, Centralbl. f. Physiol., **17**, p. 529; with Spiro, Hofmeister's Beiträge, **5**; Schittenhelm and Bodong, Arch. f. exp. Path. u. Pharm., **54**; Bordet and Gengou, Annal. Institut Pasteur, **18**. For more recent literature see Loeb, Biochem. Centralbl., **6**, p. 907. P. Nolf, Arch. internat. de Physiol., **6**, 1908.

The thrombokinase does not occur to any appreciable extent in the circulating blood, but is supplied by the form-elements. The accelerating action upon coagulation of tissues or parts of tissues depends, as above stated, upon their content of kinase; but it also in part depends upon the fact that the tissue fluids excite the secretory activity of the form-elements.

FULD [1] has arrived at about the same results independently of MORAWITZ, but he has selected other names. The three substances, thrombogen, kinase, and thrombin are called by him *plasmozym*, *cytozym*, and *holozym*. The principal reason why circulating blood remains fluid is, according to FULD, because the cytozym is only slowly formed therein and the ferment (holozym) produced thereby is quickly changed into an inactive form. Another reason is that the blood contains an antibody for the fibrin ferment. The assumption of ALEXANDER SCHMIDT that the blood contains substances retarding coagulation (anti-thrombins) has recently also received support by the observations of FULD and SPIRO, MORAWITZ, LOEB, NOLF, PUGLIESE, HOWELL [2] and others. According to HOWELL the non-coagulability of circulating blood depends on the fact that the antithrombin prevents the activation of the prothrombin into thrombin.

According to the theory of MORAWITZ, FULD and SPIRO, which is the most accepted, of those substances necessary for coagulation, only the thrombokinase (the cytozym) is absent in the circulating blood, and this is the reason why the circulating blood remains fluid. The reason why the plasma does not contain any thrombokinase lies in the fact that the healthy endothelium of the vessels does not have any irritating action upon the form-elements, and therefore no mentionable quantity of kinase is given off under these circumstances. Such an elimination occurs first outside of the blood vessels, and indeed very quickly in contact with foreign bodies. The formation of thrombin from the thrombogen takes place in an unknown manner by the action of the kinase only in the presence of lime salts (in the plasma), and this thrombin then transforms the fibrinogen into fibrin.

A serum poor in ferment and having a weak action can be reactivated by the addition of acid or alkali (ALEX. SCHMIDT, MORAWITZ), and in this action, according to MORAWITZ, a thrombin (β) is produced which is somewhat different from α-thrombin. The β-thrombin is produced from a special β-prothrombin which never occurs in the plasma, but only in the serum. FULD explains this by affirming that the α-thrombin is changed in the serum into *metazym* (β-pro-

[1] Centralbl. f. Physiol., 17. See also Fuld and Spiro, Hofmeister's Beiträge, 5.

[2] Fuld and Spiro, l. c.; Morawitz, l. c.; Loeb, Hofmeister's Beiträge, 5; Nolf, Arch. internat. de Physiol., 6; Pugliese, Biochem. Centralbl., 5, p. 930; Howell, Amer. Journ. of Physiol., 29.

thrombin), which is then transformed by the alkali or acid into *neozym* (=β-thrombin). Nevertheless it is a fact that the quantity of thrombin in the serum diminishes after coagulation, and that the thrombin action is considerably increased by the addition of alkali or acid as well as by zymoplastic substances. The above view as to the occurrence of different thrombins has not sufficient basis, and PEKELHARING [1] has also raised objections thereto.

The theories of MORAWITZ, FULD and SPIRO at least stand in accord with several known facts but do not take sufficient account of the action of the zymoplastic substances of ALEX. SCHMIDT. Thrombokinase is precipitated, by alcohol and is not thermostabile, while the zymoplastic substances, of SCHMIDT are thermostabile and soluble in alcohol. The thrombokinase cannot therefore be identical with these zymoplastic substances, and hence this theory does not explain the action of these latter. Further, the mode of action of tissue extracts is unexplained, and is a much disputed subject. It can be said that these two views are in the main opposed to each other. According to one (ALEX. SCHMIDT, ARTHUS, MORAWITZ and others) they do not act like fibrin ferment, but have an indirect action. According to the other (PEKELHARING, HUISKAMP, DELEZENNE and LOEB [2]) they are thrombin, or at least bodies having an analogous action.

CRAMER and PRINGLE [3] have made the important observation that a carefully prepared oxalate plasma when filtered through a Berkefeld filter does not coagulate on adding calcium chloride, while the unfiltered but centrifuged plasma does coagulate. The reason for this lies in the fact that centrifuged plasma contains blood-plates, which are absent in the filtered plasma. By means of these blood-plates, which yield thrombokinase, the coagulation is produced on the addition of calcium chloride. The points in NOLF's theory of coagulation that are difficult to understand as well as the observations of FREUND (page 313) and of BORDET and GENGOU (page 314) are explained by this observation.

L. LOEB,[4] who has carried out complete investigations on the coagulation of blood, especially of Crustaceæ, has arrived at the following view: The coagulation in the Crustaceæ can, according to him, be of two kinds. It may in part be an agglutination of the amœbocytes and in part a fibrin formation from a fibrinogen of the plasma. This latter coagulation is essentially the same as occurs in vertebrates. The substance acting here as the excitant for the coagulation is also active in the absence of lime salts, and behaves therefore like a thrombin. The tissues contain constituents which accelerate coagulation, which LOEB calls *coagulins*, which are not identical with the coagulins of the clot or the blood serum,

[1] Bioch. Zeitschr., **11**.

[2] Huiskamp. Zeitschr. f. physiol. Chem., **34**, 39; Delezenne, Arch. de. physiol., 1897; Loeb, Biochem. Centralbl., **6**, pages 829 and 889.

[3] Quarterly Journ. of exp. Physiol., **6**.

[4] Medical News, New York, 1903, and Virchow's Arch., **176**; Hofmeister's Beiträge, **5**, **6**, **8**, **9**, and Biochem. Centralbl., **6**, pages 829 and 889.

and these have also, although only in the presence of lime salts (if the author understands LOEB), a direct coagulating action upon fibrinogen. According to LOEB the tissue coagulins do not act as kinases in the invertebrates, and he also finds it improbable that they would act as kinases in the vertebrates. Under favorable conditions the combined blood and tissue coagulins are more active than the sum of the individual action. That this is due to an activation by a kinase, which is a possible explanation, has, in LOEB's opinion, not been proved.

The coagulins of the blood are, as above stated, according to LOEB, different from the tissue coagulins. The latter are for different classes of animals so adapted that they bring about a quicker coagulation in the blood of certain classes of animals than do the other class. The erythrocytes of mammalia (cat, dog, rabbit) contain, on the contrary, according to LOEB and FLEISHER [1] coagulins of such a specific adaptability that it is possible to differentiate between the blood corpuscles of different kinds of mammalia or, if the erythrocytes are known, to detect an unknown plasma.

Opinions are strikingly at variance in regard to the mode of action of the tissue constituents which accelerate coagulation, and their nature also is entirely unknown, hence great confusion exists on the whole in this subject.

If we accept the fact that thrombokinase does not occur in the plasma, but is produced under the influence of a foreign body acting as an excitant, it is rather difficult to understand why the plasma obtained from blood collected in a paraffined vessel and quickly and strongly centrifuged, and which is perfectly free from form-elements, should remain fluid for a long time in a paraffined vessel while it coagulates in an ordinary glass vessel. NOLF has tried by his theory to explain this difficulty, as well as the action of the alcohol-soluble zymoplastic substances (ALEX. SCHMIDT).

According to NOLF [2] the following bodies take direct part in the coagulation of the blood, namely: *Fibrinogen*, *thrombogen* (formerly called hepatothrombin by him) *thrombozym* (=thrombokinase of MORAWITZ) and *lime salts*. The coagulation of the blood, according to him, is a different process from the coagulation of a fibrinogen solution by thrombin. While in this last case the thrombin is the substance exciting coagulation, in the other case the thrombin is a product of the coagulation, as suggested by WOOLDRIDGE. In the coagulation of the plasma, according to NOLF we have a mutual precipitation of the three above-mentioned colloids—fibrinogen, thrombogen and thrombozym, all three of which are contained in the fibrin clot. This latter has correspondingly no constant composition, but varies according to the relative proportions of these three colloids. In the presence of only a little fibrinogen thrombin is produced from the three colloids (in the presence of lime salts); in the presence of abundance of fibrinogen, on the contrary, fibrin

[1] Loeb and Fleisher, Bioch. Zeitchr. **28**.

[2] Arch. internat. de Physiol., **6**, Fasc., **1**, **2**, and **3** and **7** and **9**.

is formed. Thrombin is a fibrin incompletely saturated with fibrinogen, and in the coagulation of fibrinogen with thrombin the still unsatisfied affinities of the latter are saturated. ("La thrombine d'A. Schmidt n'est-pas autre chose que de la fibrine insuffisamment pourvue de fibrinogène. Dans la coagulation du fibrinogène par la thrombine les affinités restées libres de celle-ci peuvent s'assouvir; le composé moins saturé se transforme en un composé plus saturé.") The formation of fibrin from fibrinogen is not, according to NOLF, an enzymotic process, and the thrombin is only a residue of the fibrin remaining in solution.

In NOLF'S opinion the thrombogen is probably formed in the liver and found to a large extent in all plasma. The thrombozym is secreted by the leucocytes and the endothelial cells, and in opposition to MORAWITZ is not secreted by other cells. It is also a normal constituent of the blood-plasma circulating in the living body. Most tissues, on the contrary, contain no thrombozym. The tissue extracts, NOLF believes, also contain no substances absolutely necessary for the coagulation, but only bodies which can have a powerful accelerating action, the *thromboplastic* substances which are mixed with the thrombokinase of MORAWITZ. The circulating blood-plasma contains all the bodies directly necessary in the coagulation, namely, fibrinogen, thrombogen, thrombozym and lime salts. Besides these it also contains a substance that inhibits coagulation, *antithrombin*, which is formed in the liver. There exists, if the author understands the work of NOLF, a labile equilibrium between the various constituents of the plasma, and this equilibrium is destroyed in coagulation. The first impulse to coagulation is given by the thromboplastic substances.

NOLF considers as thromboplastic active any influence of a physical or chemical nature which, be it produced by the walls of the vessel, a suspended body, a solvent or a dissolved body, a colloid or crystalloid, a molecule or an ion, makes the combination of the three above colloids possible. To the thromboplastic agents belong the walls of a glass vessel, finely powdered glass, the precipitates of calcium oxalate or calcium fluoride, also living protoplasm, aqueous tissue extracts, the alcohol soluble zymoplastic substances of ALEX. SCHMIDT, and other substances. All these agents in some way or other may serve as points of precipitation. That a plasma free from form-elements coagulates for example on contact with the walls of a glass vessel depends upon the fact that the inhibitory action of the antithrombin is retarded by the thromboplastic action of the foreign surface. Unfortunately we are not certain as to how this thromboplastic action is brought about.

An important side of NOLF'S theory of coagulation is also the *fibrinolysis* which is brought about by the thrombin. The proteolytic action of the thrombin is due only to the thrombozym contained therein, and

it has a proteolytic action only upon fibrin and not upon fibrinogen. According to NOLF, coagulation is merely a preparation for the proteolysis, and is a nutrition phenomenon, and in addition is of special importance, in arresting hemorrhage. In order to prevent a rapid fibrinolysis, the plasma also contains one or more antifibrinolytic substances, which are secreted by the liver.

What has been given contains the chief points in NOLF'S theory of coagulation, and it is impossible in a text-book to enter more into detail in regard to his remarkable investigations or the foundations on which he bases his theory and the objections which can be raised against it.

Recently other investigators as RETTGER and HOWELL have raised objections to the view that the coagulation of the blood is an enzymotic process. STROMBERG [1] also leans toward such a conception and they all raise the objection that the quantity of fibrin increases with the quantity of thrombin. This behavior, which has been known for a long time, is of such a complicated nature, that no positive conclusions can be drawn therefrom.

The belief of MELLANBY [2] that the plasma originally only contains one globulin, fibrinogen, from which by enzymotic cleavage the fibrin and serglobulin are formed, is untenable and is based upon the imperfect methods of preparing fibrinogen that he used.

From the above description of the various theories of coagulation it at least follows that in the study of the coagulation of the blood there are many contradictory statements and observations, and so many obscure points, that for the present it is impossible to give a clear, comprehensive summary of the different views and to deduce a theory of the process of coagulation which would embrace all the factors. In spite of this confusion and all contradictions, still we are sure that certain bodies such as fibrinogen and thrombin, even though this latter be an enzyme or a colloid combination, are directly concerned in the formation of fibrin, while other bodies act indirectly as accelerators or inhibitors of coagulation.

The bodies accelerating coagulation, with the exception of gelatin, whose action in this regard has not been positively proved, have been mentioned several times above. The mode of action of the bodies retarding coagulation is not clear and is much disputed. Their action may, it seems, also be more of a direct or indirect kind. Thus, for example, the oxalate and fluoride may prevent the formation of thrombin by precipitation of the lime. The cobra-poison seems to prevent the forma-

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[1] Rettger, Amer. Journ. of Physiol., **24**; Howell, *ibid.*, **26**; Stromberg, Bioch. Zeitschr., **37**.

[2] Journ. of Physiol., **38**.

tion of thrombin by the action upon the thrombokinase; the hirudin[1] may, it is generally believed, as antithrombin make the thrombin inactive, and the normal constituents of the plasma retarding coagulation perhaps act in a similar manner. In other cases the retarding bodies act indirectly, for they may, like the proteoses and others, cause the body to produce special bodies which stand in close relation to intravascular coagulation.

Intravascular Coagulation. It has been shown by ALEX. SCHMIDT and his students, as also by WOOLDRIDGE, WRIGHT,[2] and others, that an intravascular coagulation may be brought about by the intravenous injection into the circulating blood of a large quantity of a thrombin solution, as also by the injection of leucocytes or tissue fibrinogen (impure nucleoprotein). Intravascular coagulation may also be brought about under other conditions, such as after the injection of snake-poison (MARTIN[3] and others) or certain of the protein-like colloid substances, synthetically prepared according to GRIMAUX'S method (HALLIBURTON and PICKERING[4]). If too little of the above-mentioned bodies be injected, then we observe only a marked retarding tendency in the coagulation of the blood. According to WOOLDRIDGE it can generally be maintained that after a short stage of accelerated coagulability, which may lead to a total or partial intravascular coagulation, a second stage of a diminished or even arrested coagulability of the blood follows. The first stage is designated (WOOLDRIDGE) as the *positive* and the other as the *negative phase* of coagulation. These statements have been confirmed by several investigators.

There is no doubt that the positive phase is brought about by an abundant introduction of thrombin, or by a rapid and abundant formation of the same. The explanation of the production of the negative phase, which can easily be brought about by pepsin proteoses, by various bodies such as extracts of crabs' muscles and other organs, eel-serum, enzymes, bacterial toxines, certain snake-poisons, etc., has been attempted in different ways. The best studied is the action of proteoses, but no conclusive results have been obtained thus far. The assertion of PICK and SPIRO that the action of the proteoses does not depend upon the proteoses themselves, but upon a contaminating substance, the *protozym*, is claimed to be incorrect by UNDERHILL, while the recent investigations of POPIELSKI indicate that this is correct. The bodies retarding coagu-

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[1] The action of hirudin is somewhat doubtful. See Schittenhelm and Bodong, l. c.

[2] A study of the Intravascular Coagulation, etc., Proceed. of the Roy. Irish Acad. (3), 2. See also Wright, Lecture on Tissue or Cell Fibrinogen, The Lancet, 1892; and Wooldridge's Method of Producing Immunity, etc., Brit. Med. Journ., Sept., 1891.

[3] Journ. of Physiol., 15.

[4] *Ibid.*, 18.

lation, obtained by CONRADI[1] in autolysis, which are probably antithrombins, seem to act in a different way from the proteoses, and cannot for the present be made use of in explaining this question.

There are a large number of researches on the action of proteoses and of other similar retarding substances by a great number of different investigators, especially by GLEY AND PACHON, SPIRO, MORAWITZ, NOLF, DELEZENNE, DOYON and collaborators.[2] We can say with certainty that the action is indirect, and that the liver is important for the process. The non-coagulability of "peptone-blood" seems to be due to several reasons, but it has not been thoroughly explained. On the one hand such blood contains an antithrombin, and on the other it seems as if the formation of thrombin is not sufficient, although the plasma contains the necessary conditions for the thrombin formation, as it coagulates as a rule on dilution with water or the addition of a little acid. This last behavior speaks, according to MELLANBY,[3] for the assumption that the liver, because of the proteose injection, gives up an excess of alkali to the blood thus preventing the coagulation of the peptone-blood. Opinions in regard to the occurrence of an antithrombin in the peptone-plasma seem to be unanimous, and we have gained considerable experience in regard to the formation of this antithrombin. According to NOLF, the peptones (more correctly the proteoses) cause an alteration in the leucocytes and the walls of the vessels, and a substance is secreted which brings about, in the liver, the formation of antithrombin. According to DELEZENNE the proteoses bring about a destruction of leucocytes, and thereby a substance accelerating coagulation and another having a retarding action is set free. The first is destroyed by the liver, and hence the action of the retarding substance (the antithrombin) is obtained. DOYON and co-workers have also shown that the isolated washed liver on transfusing normal arterial blood, gives off a thermostable antithrombin, which behaves like a nucleoprotein. That the liver takes part in the retardation of coagulation is positively known.

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[1] Pick and Spiro, Zeitschr. f. physiol. Chem., **31**; Underhill, Amer. Journ. of Physiol., **9**; Popielski, Arch. f. expt. Path. u. Pharm., Suppl. 1908, Schmiedeberg's Festschrift; Conradi, Hofmeister's Beiträge, **1**.

[2] Grosjean, Travaux du laboratoire de L. Fredericq, **4**, Liège, 1892; Ledoux, *ibid.*, **5**, 1896; Nolf, Bull. l'Acad. roy. de Belgique, 1902 and 1905, and Biochem. Centralbl., **3**; and footnote 1, p. 318; Spiro and Ellinger, Zeitschr. f. physiol. Chem., **28**; Fuld and Spiro, l. c.; Morawitz, l. c. The works of the above-mentioned French investigators can be found in Compt. rend. soc. biol., **46**, **47**, **48**, **50**, and **51**, and Arch. d. Physiol. (5), **7**, **8**, **9**, and **10**; see also especially Delezenne, Arch. d. Physiol. (5), **10**; Compt. rend. soc. biol., **51**, and Compt. Rend., **130**; Doyon, Compt. rend. soc. biol., **68**, with Morel and Policard, *ibid*, **70**.

[3] Journ. of Physiol., **38**.

The reason of the slow coagulation of the blood in *hæmophilia* is not well known. Recent investigations of MORAWITZ and LOSSEN, SAHLI, NOLF and HENRY [1] make it very probable that the thrombokinase plays an important part. According to SAHLI the quantity of kinase is diminished, while according to NOLF and HENRY, it is qualitatively changed so that it is less active. Both cases explain the repeatedly observed relation of the vessel-walls to hæmophilia as, according to NOLF, the thrombokinase (his thrombozym) is also secreted by the endothelial cells.

The non-coagulability of cadaver blood depends usually, according to MORAWITZ,[2] upon the fact that it contains no fibrinogen, due to a fibrinolysis.

The *gases of the blood* will be treated in Chapter XVI (on respiration)

IV. THE QUANTITATIVE COMPOSITION OF THE BLOOD.

The quantitative analyses of the blood are of little value. We must ascertain on one side the relation of the plasma and blood-corpuscles to each other, and on the other the constitution of each of these two chief constituents. The difficulties which stand in the way of such a task, especially in regard to the living, non-coagulated blood, have not been removed. Since the constitution of the blood may differ not only in different vascular regions, but also in the same region under different circumstances, which renders a number of blood analyses necessary, it can hardly appear remarkable that our knowledge of the constitution of the blood is still relatively limited.

The relative volume of blood-corpuscles and serum in blood has been determined by various methods. Of these methods that of L. and M. BLEIBTREU,[3] against which important objections have been raised by several investigators, such as EYKMAN, BIERNACKI and HEDIN,[4] must be especially mentioned. In regard to this as well as to the method of St. BUGARSKY and TANGL, which is based upon a difference in the electrical conductivity of the blood and the plasma, and STEWART'S[5] colorimetric method, we must refer to the original publications.

For clinical purposes the relative volume of corpuscles in the blood may be determined by the use of a small centrifuge called a *hæmatocrit*, constructed by BLIX and described and tested by HEDIN. A measured quantity of blood is mixed with a known volume (best an equal volume)

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[1] Morawitz and Lossen, Deutsch. Arch. f. klin. Med., **94**; Sahli, *ibid.*, **99**; Nolf and Henry, Revue de médicine, **29**, 1909.

[2] Hofmeister's Beiträge, 8.

[3] Pflüger's Arch., **51**, **55**, and **60**.

[4] Biernacki, Zeitschr. f. physiol. Chem., **19**; Eykman, Pflüger's Arch., **60**; Hedin, *ibid.*, and Skand. Arch. f. Physiol., **5**.

[5] Bugarsky and Tangl, Centralbl. f. Physiol., **11**; Stewart, Journ. of Physiol., **24**.

of a fluid which prevents coagulation. This mixture is introduced into a tube and then centrifuged. According to HEDIN it is best to treat the blood, which is kept fluid by 1 p. m. oxalate, with an equal volume of a 9 p. m. NaCl solution. After complete centrifugalization, the layer of blood-corpuscles is read off on the graduated tube and the volume of blood-corpuscles (or more correctly the layer of blood-corpuscles) in 100 vols. of the blood calculated therefrom. By means of comparative counts, HEDIN and DALAND have found that an approximately constant relation exists between the volume of the layer of blood-corpuscles and the number of red corpuscles under physiological conditions, so that the number of corpuscles may be calculated from the volume. DALAND [1] has shown that such a calculation gives approximate results also in disease, when the size of the blood-corpuscles does not essentially deviate from the normal. In certain diseases, such as pernicious anæmia, this method gives such inaccurate results that it cannot be used.

KÖPPE [2] has shown that in centrifuging blood very rapidly, more than 5000 turnes per minute, the blood-corpuscles may be so completely separated that all intermediate fluid is removed. Because of the absence of this intermediate fluid the refraction is changed; the outer layers of the erythrocytes containing fat become transparent, and the column of blood-corpuscles becomes transparent and laky. If the volume of the separated column of blood-corpuscles is determined and the number of red blood-corpuscles counted, the absolute volume of these latter can be determined by this method.

In determining the relation between the weight of blood-corpuscles and the weight of blood-fluid, we generally proceed in the following manner:

If any substance is found in the blood which belongs exclusively to the plasma and does not occur in the blood-corpuscles, then the amount of plasma contained in the blood may be calculated if we determine the amount of this substance in 100 parts of the plasma or serum respectively on the one side, and in 100 parts of the blood on the other. If we represent the amount of this substance in the plasma by p and that in the blood by b, then the amount of x in the plasma from 100 parts of blood is

$$x = \frac{100.b}{p}.$$

Such a substance, which occurs only in the plasma, is fibrin according to HOPPE-SEYLER, sodium according to BUNGE (in certain kinds of blood). The experimenters just named have tried to determine the amount of the plasma and blood-corpuscles, respectively, in different kinds of blood, starting from the above-mentioned substances.

Another method suggested by HOPPE-SEYLER is to determine the total amount of hæmoglobin and proteins in a portion of blood, and on the other hand the amount of hæmoglobin and proteins in the blood-corpuscles (from an equal portion of the same blood) which have been sufficiently washed with common-salt solution by centrifugal force. The figure obtained, as a difference between these two determinations, corresponds to the amount of proteins which was contained in the serum of

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[1] Hedin, Skand. Arch. f. Physiol., **2**, 134 and 361, and **5**; Pflüger's Arch., **60**; Daland, Fortschritte, d. Med., **9**.

[2] Pflüger's Arch., **107**.

	Pig-blood.		Ox-blood.		Horse-blood.		Dog-blood.		Bull-blood.		Sheep-blood.	
	Blood-corpuscles, 435.09.	Serum, 564.91.	Blood-corpuscles, 325.5.	Serum, 674.5.	Blood-corpuscles, 397.7.	Serum, 602.3.	Blood-corpuscles, 442.8.	Serum, 577.2.	Blood-corpuscles, 334.3.	Serum, 665.7.	Blood-corpuscles, 319.2.	Serum, 680.8.
Water	272.20	518.36	192.65	616.25	243.86	551.14	277.71	514.30	206.81	608.03	200.03	624.16
Solids	162.89	46.54	132.85	58.249	153.84	51.15	165.10	42.89	127.50	57.66	118.82	56.63
Hæmoglobin	142.20	—	103.10	—	125.8	—	145.6	—	106.40	—	102.80	—
Protein	8.35	38.26	20.89	48.901	20.05	42.65	2.36	34.05	15.38	46.41	12.80	46.56
Sugar	—	0.684	—	0.708	—	0.90	—	0.74	—	0.679	—	0.708
Cholesterin	0.213	0.231	1.100	0.835	0.26	0.31	0.56	0.37	0.610	0.599	1.147	0.891
Lecithin	1.504	0.805	1.220	1.129	1.93	1.05	1.02	0.98	0.953	1.244	1.329	1.088
Fat	—	1.104	—	0.625	—	0.50	—	0.91	—	2.357	—	0.859
Fatty acids	0.027	0.448	—	—	0.02	0.36	—	0.70	—	0.494	—	0.4908
Phosphoric acid as nuclein	0.0455	0.0123	0.0178	0.0089	0.05	0.01	0.05	0.01	0.0194	0.0089	0.0235	0.0109
Soda	—	2.401	0.7266	2.9084	—	2.62	1.27	2.39	0.839	2.873	0.760	2.917
Potash	2.157	0.152	0.2351	0.1719	1.32	0.15	0.11	0.14	0.233	0.174	0.236	0.172
Iron oxide	0.696	—	0.544	—	0.59	—	0.71	—	0.562	—	0.545	—
Lime	—	0.0689	—	0.0805	—	0.07	—	0.06	—	0.073	—	0.089
Magnesia	0.0656	0.0233	0.0056	0.0300	0.04	0.03	0.03	0.03	0.009	0.027	0.006	0.027
Chlorine	0.642	2.048	0.5901	2.4889	0.18	2.20	0.60	2.31	0.628	2.453	0.575	2.516
Phosphoric acid	0.8956	0.1114	0.2392	0.1646	0.98	0.15	0.67	0.14	0.236	0.156	0.228	0.163
Inorganic P_2O_5	0.7194	0.0296	0.1140	0.0571	0.76	0.05	0.54	0.05	0.133	0.041	0.088	0.057

	Goat-blood.		Cat-blood.		Rabbit-blood.		Human Blood, Man.		Human Blood, Woman.	
	Blood-corpuscles, 347.2.	Serum, 652.8.	Blood-corpuscles. 434.0.	Serum, 566.0.	Blood-corpuscles, 372.1.	Serum, 627.9.	Blood-corpuscles, 513.02.	Serum, 486.98.	Blood-corpuscles, 396.24.	Serum, 603.76.
Water	211.35	592.54	270.90	524.17	235.74	518.18	349.69	439.02	272.56	551.99
Solids	135.86	60.25	163.11	41.35	136.37	46.71	163.33	47.96	123.68	51.77
Hæmoglobin	112.50	—	143.2	—	123.50	—				
Protein	18.76	50.96	11.62	33.16	4.55	33.63				
Sugar	—	0.822	—	0.860	—	1.036	Organic			
Cholesterin	0.601	0.698	0.556	0.339	0.268	0.343	bodies			
Lecithin	1.339	1.127	1.354	0.971	1.722	1.105	159.59	43.82	120.13	46.70
Fat	—	0.0407	—	0.446	—	0.749				
Fatty acids	—	0.398	—	0.282	—	0.507				
Phosphoric acid as nuclein	0.028	0.0117	0.063	0.009	0.040	0.015	Inorg. 3.74	4.14	3.55	5.07
Soda	0.755	2.824	1.174	2.512	—	2.789	0.24	1.66	0.65	1.92
Potash	0.236	0.160	0.112	0.148	1.946	0.162	1.59	0.15	1.41	0.20
Iron oxide	0.547	—	0.694	—	0.615	—	—	—	—	—
Lime	—	0.078	—	0.062	—	0.072	—	—	—	—
Magnesia	0.014	0.026	0.035	0.024	0.029	0.028	—	—	—	—
Chlorine	0.514	2.409	0.455	2.360	0.460	2.438	0.90	1.72	0.36	0.14
Phosphoric acid	0.243	0.154	0.697	0.133	0.835	0.151	—	—	—	—
Inorganic P_2O_5	0.097	0.045	0.515	0.040	0.645	0.040	—	—	—	—

the first portion of blood. If we now determine the proteins in a special portion of serum of the same blood, then the amount of serum in the blood is easily determined. The usefulness of this method has been confirmed by BUNGE by the control experiments with sodium determinations. If the amount of serum and blood-corpuscles in the blood is known, and we then determine the amount of the different blood-constituents in the blood-serum on one side and of the total blood on the other, the distribution of these different blood-constituents in the two chief components

of the blood, plasma and blood-corpuscles may be ascertained. In the table on page 328 are given analyses of the blood of various animals by ABDERHALDEN [1] according to HOPPE-SEYLER'S and BUNGE'S methods. The analyses of human blood by C. SCHMIDT [2] are older and were made according to another method, hence the results for the weights of the corpuscles are perhaps a little too high. All the results are in parts per 1000 parts of blood.

The relation between blood-corpuscles and plasma may vary considerably under different circumstances even in the same species of animal. In animals, in most cases considerably more plasma is found, sometimes two-thirds of the weight of the blood.[3] For human blood ARRONET has found 478.8 p. m. blood-corpuscles and 521.2 p.m. serum (in defibrinated blood) as an average of nine determinations. SCHNEIDER [4] found 349.6 and 650.4 p. m. respectively in women.

The sugar was considered as occurring only in the serum and not with the blood-corpuscles. According to the investigations of RONA and MICHAELIS the blood-corpuscles of the dog contain considerable amounts of sugar; and the quantity of sugar in the blood, in the blood-corpuscles as well as in the plasma, is increased in man with diabetes mellitus. HOLLINGER [5] also found that in man, with normal quantity of sugar in the blood, the sugar was distributed almost equally between the blood-corpuscles and the plasma.

The amount of sugar in the blood-corpuscles, which was shown by LÉPINE and BOULUD before MICHAELIS and RONA, has been the subject of numerous investigations by BANG and his pupils, LYTTKENS and SANDGREN on the one hand and by RONA, MICHAELIS, TAKAHASHI, FRANK and others on the other hand [6]. The results of these investigations are so contradictory that it is hardly possible for the present to draw any positive conclusions. It seems to follow from them, nevertheless, that the dog blood-corpuscles always contain sugar, while for the corpuscles of the rabbit and man the conditions are somewhat doubtful and may be variable (FRANK and BRETSCHNEIDER). According to LYTTKENS and SANDGREN the blood-corpuscles of man contain as maximum 0.06

[1] Zeitschr. f. physiol. Chem., **23** and **25**.

[2] Cited and in part recalculated from v. Gorup-Besanez, Lehrb. d. physiol Chem., 4. Aufl., 345.

[3] See Sacharjin in Hoppe-Seyler's Physiol. Chem., 447; Otto, Pflüger's Arch., **35**; Bunge, Zeitschr. f. Biol., **12**; L. and M. Bleibtreu, Pflüger's Arch., **51**.

[4] Arronet, Maly's Jahresber., **17**; Schneider, Centralbl, f. Physiol., **5**, 362.

[5] Rona and Michaelis, Bioch. Zeitschr. **16** and **18**; Hollinger, *ibid.*, **17**.

[6] Lépine and Boulud, Bioch. Zeitschr., **32**; Lyttkens and Sandgren, *ibid.*, **26**, **31**, **36**; Rona with Döblin, *ibid.*, **31**, with Michaelis, *ibid.*, **37**, with Takahashi, *ibid.*, **30**; Takahashi, *ibid.*, **37**; E. Frank, Zeitschr. f. physiol. Chem., **70**, with Bretschneider, *ibid.*, **71** and **76**; see also Oppler, *ibid.*, **64** and **75**.

p. m. sugar. The blood-corpuscles of the ox, sheep, horse, pig, cat and guinea-pig do not contain any sugar according to these last-mentioned investigators. On the contrary the blood-plasma as well as the blood-corpuscles contain a non-fermentable reducing substance. The quantity of this in the human blood-corpuscles is 0.6 p. m. according to LYTTKENS and SANDGREN and in the blood-corpuscles of different animals an average of 0.44–0.8 p. m. calculated as glucose. The quantity of the non-fermentable bodies in the blood-plasma of the animals investigated by them was 0.3 to 0.5 p. m.

The quantity of glucose in the blood cannot be exactly determined. As the blood also contains other reducing substances besides glucose the total reduction naturally cannot be used as an exact value for the glucose content; and it must also be added that the different methods do not give uniform results. Thus on using the methods of KNAPP and BANG, which give the total reduction, higher values are obtained than with ALLIHN'S or BERTRAND'S methods, in which the quantity of precipitated cuprous oxide is determined. The polarization method cannot give exact results because of the presence of other optically active substances and objections can also be raised against the fermentation method.[1] On using this last method OTTO[2] first observed, and was substantiated later by others, namely BANG and his co-workers, that the blood contained non-fermentable bodies which reduced KNAPP'S (and also BANG'S) solution. The remaining reduction "rest reduction" after the fermentation cannot be detected according to BERTRAND'S titration method.

The nature of this reducing but not fermentable substance occurring in the plasma as well as in the blood-corpuscles is not known. The assumption of JACOBSEN, BING, and HENRIQUES[3] that this questionable substance is jecorin or lecithin sugar does not have sufficient foundation, and the question of the identity with jecorin is doubtful and is connected with the question as to the existence of jecorin at all. The conjugated glucuronic acids have also been considered and according to the investigations of MAYER, LÉPINE and BOULUD[4] they occur in blood and originate in the form-elements. For these assumptions we do not have sufficient support, and especially we have no explanation

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[1] In regard to methods see Bang, Der Blutzucker, Wiesbaden, 1913 which also describes a new method suggested by him for the determination of sugar in very small amounts of blood.

[2] Pflüger's Arch., **35**.

[3] Jacobson, Centralbl. f. physiol. **6**; Bing, Skand. Arch. f. physiol., **9**; Henriques, Zeitschr. f. physiol Chem., **23**. See also P. Mayer, Bioch. Zeitschr., **1** and **4**.

[4] Mayer, Zeitschr. f. physiol. Chem., **32**; Lépine and Boulud, Compt. Rend., **133**, **135**, **136**, **138**, **141** and Journ. de Physiol., **7** (cited from Bioch. Centralbl., **4**, page 421).

for the total rest reduction. FRANK and BRETSCHNEIDER[1] have, nevertheless, shown that the reducing substance or mixture that occurs in the blood-corpuscles, and which does not reduce BERTRAND'S solution, but does reduce BANG'S solution, yields a reduceable sugar on boiling with acid which now reduces BERTRAND'S solution. The corresponding substance in the blood-plasma has a similar behavior. If, as in the experiments of FRANK and BRETSCHNEIDER, the extent of reduction after acid hydrolysis is about the same as the original substance (titrated according to BANG) we cannot here be dealing with dextrins and the nature of this body in question (or mixture) is quite unknown.

In close relation to what has been given above is the question of "sucre immediat" and the "sucre virtuel" of LÉPINE and BOULUD.[2] They designate as "sucre immediat" the reduction, calculated as sugar, of the blood immediately after leaving the blood vessels and as "sucre virtuel" the increase in the reducing power brought on in part by allowing the blood to stand after leaving the body, in part by the action of invertase or emulsin at 39° C. and in part by boiling with hydrofluoric acid. The quantity of "sucre virtuel" in dogs amounts to an average of 70 per cent of the "sucre immediat." The nature of the "sucre virtuel" is not well known; from what was said above we are probably dealing here to all appearances with very different bodies.

From what has been presented above it can be understood why the exact sugar content of the blood is not known. In consideration of the above mentioned difficulties and sources of error attempts have been made to determine the sugar content of the blood and we will give the results of some of these.

The quantity of actual sugar in the blood, amounts according to LYTTKENS and SANDGREN, in man to 0.63, in sheep 0.64, pig 0.82, ox 0.86, horse 0.98, rabbit 2.22, guinea-pig 2.48 and in the cat 2.91 p. m. Small animals with an active metabolism contain more sugar in the blood than larger animals. According to FRANK the amount of sugar in the blood-plasma of man lies between 0.8 and 1.1 p. m. and according to FRANK and COBLINER[3] it is 1.19–1.26 p. m. in new-born.

The amount of blood sugar seems to be almost independent of the character of the food. After feeding with large amounts of sugar or dextrin, BLEILE, nevertheless, has observed a considerable increase in the sugar. The amount of sugar is not only somewhat different with various animals but it also varies with the same animal under different

[1] Zeitschr. f. physiol. Chem., **71** and **76**.

[2] Compt. Rend., **137**, **144**, **147**, and Journ de Physiol. et d. Path., **11** and **18**.

[3] Lyttkens and Sandgren, Bioch. Zeitschr., **36**; Frank and Cobliner, Zetischr. f. physiol. Chem., **70**.

external conditions. When it amounts to more than 3 p. m., according to a statement of Cl. BERNARD,[1] sugar appears in the urine and a glycosuria occurs, a view that has not been substantiated. On the one hand a glycosuria may occur at a lower sugar content in the blood and on the other hand a glycosuria may be absent for a time with a higher sugar content. An increase in the sugar content occurs, as first shown by BERNARD and subsequently proved by others, after drawing blood. In this case not alone is the quantity of sugar increased but also the other reducing substances. According to certain investigators the quantity of these latter is especially increased (HENRIQUES, N. ANDERSON, LYTTKENS and SANDGREN, LÉPINE and BOULUD [2]).

BERNARD [3] has shown that the quantity of sugar in the blood diminishes more or less rapidly on leaving the veins. LÉPINE, associated with BARRAL, has specially studied this decrease in the quantity of sugar, and calls it *glycolysis*. LÉPINE and BARRAL, as well as ARTHUS, have shown that this glycolysis takes place in the complete absence of micro-organisms. It seems to be due to a soluble *glycolytic enzyme* whose activity is destroyed by heating to 54° C. This enzyme is derived, according to the above investigators, from the leucocytes and, according to ARTHUS as well as to DOYON and MOREL [4] it occurs only in the serum but not in the plasma. According to LÉPINE,[5] it has some connection with the pancreas. The glycolysis is, according to RÖHMANN and SPITZER and SIEBER, an oxidation which is produced, according to the two last-mentioned investigators, by an oxidation ferment. According to RONA and DÖBLIN it takes place in an atmosphere of hydrogen, which does not speak for the above view. The recent investigations of SLOSSE, of EMBDEN and collaborators KRASKE, KONDO and K. v. NOORDEN [6]

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[1] Bleile, Arch. f. (Anat. u.) Physiol., 1879; Bernard, Leçons sur le diabète.

[2] Henriques, Zeitschr. f. physiol. Chem., **23**, N. Anderson, Bioch. Zeitschr., **12**; Lyttkens and Sandgren, *ibid.*, **26**; Lépine and Boulud, Journ. de Physiol., **13**.

[3] Leçons sur le diabète, Paris, 1877.

[4] Arthus, Arch. de Physiol. (5), **3**; Doyon and Morel, Compt. rend soc. biol., **55**.

[5] In regard to the numerous memoirs of Lépine and Lépine and Barral, see Lyon médical., **62** and **63**; Compt Rend. **110**, **112**, **113**, **120** and **139**; Lépine, Le ferment glycolytique et la pathogénie du diabète (Paris, 1891), and Revue analytique et critique des travaux, etc., in Arch. de méd. expér. (Paris, 1892); Revue de médecine 1895; État actuel de la question de la glycolyse, Semaine médicale, 1911; Arthus, Arch. de Physiol (5), **3**, 4; Nasse and Framm, Pflüger's Arch., **63**, Paderi, Maly's Jahresber., **26**; see also Cremer, Physiologie des Glykogens in Ergebnisse d. Physiol., **1**, Abt. 1.

[6] Röhmann and Spitzer, Ber. d. d. chem. Gesellsch., **28**; Spitzer, Pflüger's Arch., **60** and **67**; Sieber, Zeitschr. f. physiol Chem., **39** and **44**; Rona and Döblin, Bioch. Zeitschr., **32**; Slosse, Arch. internat. de Physiol., **11**; Kraske, Kondo, and v. Noorden Bioch. Zeitschr., **45**.

speak positively for the statement that in glycolysis a formation of lactic acid from the sugar occurs.

That a formation of lactic acid from glucose, and indeed by means of the leucocytes, takes place in glycolysis was shown by LEVENE and MEYER before EMBDEN and collaborators. On continuing these investigations LEVENE and MEYER found that fructose as well as mannose and galactose under the same conditions with leucocytes, yield *d*-lactic acid while with the investigated pentoses, arabinose and xylose, this is not the case. According to EMBDEN and co-workers, this formation of lactic acid takes place probably with glyceric aldehyde, and perhaps also with small amounts of dioxyacetone, as intermediary steps, and a formation of lactic acid from glyceric aldehyde (and dioxyacetone) can in fact, as A. LOEB and GRIESBACH [1] have shown, be brought about by enzymotic means by the form-elements of the blood. It seems as if several enzymes were active in the formation of lactic acid from glucose. According to LOEB those varieties of blood which show no glycolysis with the formation of lactic acid, or none worth mentioning, can form lactic acid from glyceric aldehyde and according to GRIESBACH in this last-mentioned process an enzyme is active which is soluble in water and resistant toward the hæmolysis of the blood with water, while the action of the blood upon glucose is destroyed in the destruction of the form-elements by hæmolysis. In regard to the formation of lactic acid from methyl glyoxal see page 584. According to LÉPINE and BOULUD a double process takes place in the glycolysis. On one side the sugar is destroyed and on the other side a re-formation of sugar from the "sucre virtuel" takes place. Hereby the actual glycolysis may be greater than the visible, and the mentioned investigators have therefore suggested a method for determining the extent of the actual glycolysis.[2]

The quantity of urea, which, according to SCHÖNDORFF, is equally divided between the blood-corpuscles and the plasma, is greater on taking food than in starvation (GRÉHANT and QUINQUAUD, SCHÖNDORFF) and varies between 0.2 and 1.5 p. m. In dogs SCHÖNDORFF found in starvation a minimum of 0.348 p. m. and a maximum of 1.529 p. m. at the point of highest urea formation. GOTTLIEB obtained much lower results by another direct method, namely, in starvation 0.1–0.2, and after meat feeding 0.28–0.56 p. m., FOLIN and DENIS found 0.3–0.77 p. m. in the blood of the cat. In man v. JAKSCH [3] found 0.5–0.6 p. m.

[1] Levene and Meyer, Journ. of biol. Chem., **11** and **14**; A. Loeb, Bioch. Zeitschr. **49** and **50**; Griesbach, *ibid.*, **50**.

[2] Lépine and Boulud, Journ. de Physiol., et de Path. générale, **13**.

[3] Gréhant et Quinquaud, Journ. de l'anatomie et de la physiol., **20**, and Compt. Rend., **98**; Schöndorff, Pflüger's Arch., **54** and **62**; Gottlieb, Arch. f. exp. Path. u. Pharm., **42**; Folin and Denis, Journ. of biol. Chem., **11** and **12**; v. Jaksch, Leyden-Festschr., I, 1901.

urea in normal blood. The quantity of urea is somewhat increased in fever, and in general in augmented protein metabolism the increased urea formation is dependent upon this. A more important increase in the quantity of urea in the blood occurs in a retarded elimination of urea, as in cholera, also in cholera infantum, and in infections of the kidneys and urinary passages. After ligaturing the ureters or after extirpation of the kidneys of animals, an accumulation of urea takes place in the blood.

v. SCHRÖDER first showed that the blood of the shark was very rich in urea, and the quantity indeed amounted to 26 p. m. BAGLIONI[1] has recently shown that this large quantity of urea is of the greatest importance, as the presence of urea in these animals is a necessary life-condition for the heart and very probably for all organs and tissues.

The blood also contains traces of ammonia. According to HORODYNSKI, SALASKIN, and ZALESKI,[2] the quantity in arterial dog-blood was 0.41 milligram in 100 grams of blood. According to WINTERBERG,[3] the blood from healthy persons contains on an average 0.90 milligram per 100 cc.[3] The quantity of uric acid may be 0.1 p. m. in bird's blood (v. SCHRÖDER[4]). Uric acid has only recently been positively detected under normal conditions, while it has been found, earlier, in the blood in gout, croupous pneumonia, and certain other diseased conditions. FOLIN and DENIS[5] have determined the uric acid in the blood of certain animals as well as in man by a colorimetric method suggested by FOLIN. Normal human blood contains not less than 1 to 2–2.5 milligrams uric acid per 100 grm.; in gout they found 5.5 milligrams as maximum. They also determined the quantity of total non-protein nitrogen and urea nitrogen in human blood. In normal blood the first was equal to 22–26 milligrams and the last equal to 11–13 (=24–28 urea) milligrams in 100 grams of blood. In disease great variations were found. Lactic acid was first found in human blood by SOLOMON and then by GAGLIO, BERLINERBLAU, and IRISAWA. The quantity of lactic acid may vary considerably. BERLINERBLAU found 0.71 p. m. as maximum, in dog's blood. SAITO and KATSUYAMA[6] found on an average 0.269 p. m. in hen's blood, and after carbon-monoxide poisoning the quantity increased to 1.227 p. m. Fat and fatty acids occur

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[1] v. Schröder, Zeitschr. f. physiol. Chem., **14**; Baglioni, Centralbl. f. Physiol., **19**.

[2] Zeitschr. f. physiol. Chem., **35**, which also gives the older literature.

[3] Wien. klin. Wochenschr., 1897, and Zeitschr. f. klin. Med., **35**.

[4] Ludwig's Festschrift, 1887.

[5] Journ. of biol. Chem., **13** and **14**.

[6] Irisawa, Zeitschr. f. physiol. Chem., **17**, which also gives the older literature; Saito and Katsuyama, *ibid.*, **32**.

perhaps only in the serum. The small traces of bile acids occurring in normal blood, according to CROFTAN,[1] are contained in the leucocytes.

The calcium occurs, with the exception perhaps of the blood corpuscles of the ox, only in the plasma and the same applies at least for the principal part of the magnesium. The division of the alkali between the blood-corpuscles and the plasma is very different, namely, the blood-corpuscles of the pig, horse and rabbit contain no sodium, the human corpuscles are richer in potassium and those of the ox, sheep, goat, dog and cat are much richer in sodium than potassium. Chlorine occurs in greater abundance in the serum of all animals than in the blood-corpuscles. The iodine only occurs in serum, while iron regularly, almost without exception occurs in the form-elements, especially in the erythrocytes. As the nucleoproteins contain iron, some iron occurs in the leucocytes and traces of iron also occur in the serum. This quantity is very small under normal conditions while in disease the relationship between the hæmoglobin-iron and the other blood-iron may, it seems, changes very distinctly. Manganese has also been found in the blood, as well as traces of lithium copper, lead, silver, and also arsenic in menstrual blood. The entire blood contains in ordinary cases 770–820 p. m. water with 180–230 p. m. solids, among these 173–220 p. m. are organic and 6–10 p. m., inorganic. The organic consist, after substracting 6–12 p. m. extractives, of protein and hæmoglobin. The quantity of the latter in man is 130–150 p. m. In the dog, cat, pig and horse the hæmoglobin content is about the same; in ox, bull, sheep, goat and rabbit blood it is lower (ABDERHALDEN).

The Composition of the Blood in Different Vascular Regions and under Different Conditions.

Arterial and Venous Blood. The most striking difference between these two kinds of blood is the variation in color caused by their containing different amounts of gas and different amounts of oxyhæmoglobin and hæmoglobin. The arterial blood is light red; the venous blood is dark red, dichroic, greenish by transmitted light through thin layers. The arterial coagulates more quickly than the venous blood. The latter, on account of the transudation which takes place in the capillaries, was formerly said to be somewhat poorer in water but richer in blood-corpuscles and hæmoglobin than the arterial blood; but this is denied by modern investigators. According to KRÜGER[2] and his pupils the quan-

[1] Pflüger's Arch., 90.

[2] Zeitschr. f. Biologie, 26. This also gives the literature on the composition of the blood in different vascular regions.

tity of dry residue and hæmoglobin in blood from the carotid artery and from the jugular vein (in cats) is the same. RÖHMANN and MÜHSAM could not detect any difference in the quantity of fat in arterial and venous blood. The serum from dog's blood has, according to WIENER,[1] a relatively higher globulin content relative to the albumin in the venous blood as compared with the arterial blood.

Blood from the Portal Vein and the Hepatic Vein. In consequence of the small quantities of bile and lymph formed relatively to the large quantity of blood circulating through the liver in a given time, we can hardly expect to detect by chemical analysis a positive difference in the composition between the blood of the portal and hepatic veins. The statements in regard to such a difference are in fact contradictory. For example, DROSDOFF found more hæmoglobin in the hepatic than in the portal vein, while OTTO found less. KRÜGER finds that the quantities of hæmoglobin, as well as of the solids, in the blood from the vessels passing to and from the liver are different, but a constant relation cannot be determined. The hepatic vein, according to DOYON and collaborators,[2] is richer in fibrinogen than the blood from the portal vein. The disputed question as to the varying quantities of sugar in the portal and hepatic veins will be discussed in a following chapter (see Chapter VII, on the formation of sugar in the liver). After a meal rich in carbohydrates, the blood of the portal vein not only becomes richer in glucose, but may also contain dextrin and other carbohydrates (v. MERING, OTTO[3]). The amount of urea in the blood from the hepatic vein is greater than in other blood (GRÉHANT and QUINQUAUD). In portal blood FOLIN and DENIS found about the same amount of urea as in the carotid blood. Like HORODJNSKI, SALASKIN and ZALESKI,[4] they found that the portal blood was richer in ammonia than the carotid blood. The largest amount of ammonia was always found in the blood from the mesentery vessels of the large intestine.

Blood of the Splenic Vein is decidedly richer in leucocytes than the blood from the splenic artery. The red blood-corpuscles of the blood from the splenic vein are smaller than the ordinary, are less flattened, and show a greater resistance to water. The blood from the splenic vein is also claimed to be richer in water, fibrin, and protein than the ordinary venous blood. According to v. MIDDENDORFF, it is richer in hæmoglobin

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[1] Röhmann and Mühsam, Pflüger's Arch., **46**; Wiener, Zeitschr. f. physiol. Chem., **32**.

[2] See footnote 2, page 253.

[3] Drosdoff, Zeitschr. f. physiol. Chem., **1**; Otto, Maly's Jahresber, **17**; v. Mering, Arch. f. (Anat. u.) Physiol., 1877, 214.

[4] Gréhant et Quinquaud, l. c. footnote 3, page 333; Folin and Denis, Journ. of biol. Chem., **11** and **12**; Horodjnski, Salaskin and Zaleski, Zeitschr. f. physiol. Chem., **35**.

than arterial blood. KRÜGER[1] and his pupils found that the blood from the vana lienalis is generally richer in hæmoglobin and solids than arterial blood; still the contrary is often found. The blood from the splenic vein coagulates slowly.

The Blood from the Veins of the Glands. The blood circulates with greater rapidity through a gland during activity (secretion) than when at rest, and the outflowing venous blood has therefore during activity a lighter red color and a greater amount of oxygen. Because of the secretion, the venous blood also becomes somewhat poorer in water and richer in solids.

The blood from the *Muscular Veins* shows an opposite behavior, for during activity it is darker and more venous in its properties because of the increased absorption of oxygen by the muscles and still greater production of carbon dioxide than when at rest.

Menstrual Blood, according to an old belief, has not the power of coagulating. This statement, is nevertheless, false, and the apparent uncoagulability depends in part on the retarding action of the mucous membrane of the uterus upon coagulation (CRISTEA and DENK[2]) and in part on a contamination with vaginal mucus, which disturbs the coagulation. Menstrual blood, according to GAUTIER and BOURCET, contains arsenic and is also richer in iodine than other blood (see Blood-serum, page 269).

The Blood of the Two Sexes. Women's blood coagulates somewhat more quickly, has a lower specific gravity, a greater amount of water, and a smaller quantity of solids than the blood of man. The amount of blood-corpuscles and hæmoglobin is somewhat smaller in woman's blood. The amount of hæmoglobin is 146 p. m. for man's blood and 133 p. m. for woman's.

During *pregnancy* NASSE has observed a decrease in the specific gravity, with an increase in the amount of water, until the end of the eighth month. From then the specific gravity increases, and at delivery it is again normal. The amount of fibrin is somewhat increased (BECQUEREL and RODIER, NASSE). The number of blood-corpuscles seems to decrease. In regard to the amount of hæmoglobin the statements are somewhat contradictory. COHNSTEIN found the number of red corpuscles diminished in the blood of pregnant sheep as compared with non-pregnant, but the red corpuscles were larger and the quantity of hæmoglobin in the blood was greater in the first case. MÖLLENBERG found in most cases an increase in the amount of hæmoglobin in pregnancy in the last months,

[1] v. Middendorff, Centralbl. f. Physiol., 2, 753; Krüger, l. c.

[2] Cristea and Denk, Maly's Jahresb., 40, 181.

and according to HERMANN and NAUMANN [1] an increase in the cholesterin ester and the neutral fats occurs in the blood during pregnancy.

The Blood at Different Periods of Life. Fetal and infant blood is richer in erythrocytes and hæmoglobin than the blood of the mother. In animals this is true at least for the hæmoglobin while the number of erythrocytes in growing or adult animals may be greater than in new-born animals. The highest percentage of hæmoglobin in the blood has been observed by several investigators, such as COHNSTEIN and ZUNTZ, OTTO, WINTERNITZ, ABDERHALDEN, SCHWINGE, and others, immediately or very soon after birth or at least within the first few days. In man two or three days after birth the hæmoglobin reaches a maximum (200–210 p. m.) which is greater than at any other period of life. This is the cause of the great abundance of solids in the blood of new-born infants, as observed by several investigators. The quantity of hæmoglobin and blood-corpuscles sinks gradually from this first maximum to a minimum of about 110 p. m. hæmoglobin, which minimum appears in human beings between the fourth and eighth years. The quantity of hæmoglobin then increases again until about the twentieth year, when a second maximum of 137–150 p. m. is reached. The hæmoglobin remains at this point only to about the forty-fifth year, and then gradually and slowly decreases (LEICHTENSTERN, OTTO [2]). According to earlier reports, the blood at old age is poorer in blood-corpuscles and protein bodies, but richer in water and salts.

The Influence of Food on the Blood. In complete starvation no decrease in the amount of solid blood-constituents is found to take place (PANUM and others). The amount of hæmoglobin is increased a little, at least in the early period (SUBBOTIN, OTTO, HERMANN and GROLL, LUCIANI and BUFALINI), and also the number of red blood-corpuscles increases (WORM MULLER, BUNTZEN [3]), which probably depends partly on the fact that the blood-corpuscles are not so quickly transformed as the serum and partly on a greater concentration due to loss of water.

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[1] Nasse, Maly's Jahresber., 7; Becquerel and Rodier, Traité de chim. pathol., Paris, 1854; Cohnstein, Pflüger' Arch., 34, 233; Möllenberg, Maly's Jahresber., 31, 185. See also Payer, Arch. f. Gynäk., 71; Herrmann and Naumann, Bioch. Zeitschr., 43.

[2] Cohnstein and Zuntz, Pflüger's Arch., 34; Winternitz, Zeitschr. f. physiol. Chem., 22; Leichtenstern, Untersuch. über. den Hämoglobingehalt des Blutes, etc., Leipzig, 1878; Otto, Maly's Jahresber., 15 and 17; Abderhalden, Zeitschr. f. physiol. Chem., 34; Schwinge, Pflüger's Arch., 73 (literature). See also Fehrsen, Journ. of Physiol., 30.

[3] Panum, Virchow's Arch., 29; Subbotin, Zeitschr. f. Biologie, 7; Otto. l. c., Worm Müller, Transfusion und Plethora, Christiania, 1875; Buntzen, see Maly's Jahresber., 9; Hermann and Groll, Pflüger's Arch., 43; Luciani and Bufalini, Maly's Jahresber., 12.

In rabbits and to a less extent in dogs, POPEL found that complete abstinence had a tendency to increase the specific gravity of the blood. The amount of fat in the blood may be somewhat increased in starvation because the fat is taken up from the fat deposits and carried to the various organs by the blood (N. SCHULZ, DADDI [1]).

After a rich meal, or after secretion of digestive juices or absorption of nutritive liquids, the relative number of blood-corpuscles may be increased or diminished (BUNTZEN, LEICHTENSTERN). The number of white blood-corpuscles may be considerably increased after a diet rich in proteins. After a diet rich in fat the plasma becomes, even after a short time, more or less milky-white, like an emulsion. According to JUST, in rabbits, on the contrary, the various food-stuffs such as carbohydrate, fat and protein or peptone has no influence on the number of red and white corpuscles, which he considers as a proof for the difference between the digestive processes in carnivora and herbivora (rabbits). The composition of the food acts essentially on the amount of hæmoglobin in the blood. SUBBOTIN has observed in dogs after a one-sided feeding with food rich in carbohydrates that the amount of hæmoglobin sank, from the physiological average of 137.5 p. m. to 103.2–93.7 p. m. TSUBOI [2] has also shown in experiments on rabbits and dogs that with an insufficient diet of bread and potatoes, where the body gave up protein and contained relatively considerable carbohydrate, the amount of hæmoglobin decreased and the blood became richer in water. According to LEICHTENSTERN, a gradual increase in the amount of hæmoglobin is found to take place in the blood of human beings on enriching the food, and according to the same investigator the blood of lean persons is generally somewhat richer in hæmoglobin than blood from fat ones of the same age. The addition of iron salts to the food greatly influences the number of blood-corpuscles and especially the amount of hæmoglobin they contain. The action of the iron salts is obscure.[3] There does not seem to be any doubt that the iron contained in the food in the form of organic compounds is active, but also iron salts and therapeutic iron. According to BUNGE and his pupils the iron preparations act indirectly only. They may combine with the sulphureted hydrogen of the intestinal canal and thereby prevent the iron associated in the food as assim-

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[1] Popel, Arch. des scienc. biol. de St. Pétersbourg, **4**, 354; Schulz, Pflüger's Arch., **65**; Daddi, Maly's Jahresber., **30**.

[2] Just, Centralbl. f. Physiol., **23**; Subbotin, l. c.; Tsuboi, Zeitschr. f. Biologie, **44**.

[3] See Bunge, Zeitschr. f. physiol. Chem., **9**; Häusermann, *ibid.*, **28**, where the works of Woltering, Gaule, Hall, Hochhaus, and Quincke are cited (the same work contains a table of the quantity of iron in various foods); Kunkel, Pflüger's Arch., **61**; Macallum, Journal of Physiol., **16**; Abderhalden, Zeitschr. f. Biologie, **39**.

ilable protein compounds from being eliminated as iron sulphide (BUNGE), a view which is now generally discarded.

An *increase in the number of red corpuscles*, takes place after transfusion of blood of the same species of animal. According to the observations of PANUM and WORM MÜLLER,[1] the blood-liquid is quickly eliminated and transformed in this case—the water being eliminated principally by the kidneys and the protein burned into urea, etc.—while the blood-corpuscles are preserved longer and cause a "*polycythæmia.*" A relative increase in the number of red corpuscles is found after abundant transudation from the blood, as in cholera and heart-failure with considerable congestion. An increase in the number of red blood-corpuscles has also been observed under the influence of diminished pressure or in high altitudes. VIAULT first called attention to the fact that the number of red corpuscles was very great in the blood of man and animals living in high regions. According to him the llama has about 16 million blood-corpuscles per cubic millimeter. By observations on himself and others, as well as on animals, VIAULT found the first effect of sojourning in high altitudes was a very considerable increase in the number of red corpuscles, in his own case 5–8 millions. In a young man residing for four weeks in 2900 meters altitude, LACQUEUR observed an increase in the erythrocytes as well as the hæmoglobin in the unit volume of the blood. The maximum, which appeared first after 15 days, was 15 per cent increase for the erythrocytes and 16 per cent for the hæmoglobin. He also found that dogs from whom about one-half of the blood was drawn required at 2900 meters altitude, on an average of 16 days to replace the same, while at the normal level this requires on an average of 27 days or in round numbers an increase of 70 per cent. Both observations show a re-formation of blood under the influence of high altitude. COHNHEIM and WEBER[2] found in 23 men engaged on the Jungfrau railroad, who had lived for a long time in high altitudes that the number of erythrocytes (5.2–6.3 million) as well as the hæmoglobin (generally 87–94 as compared to 80 per cent as normal) was higher than under normal conditions, and they consider this as a proof for the actual formation of blood-corpuscles in high altitudes. A similar increase of the red blood-corpuscles, as also an increase in the quantity of hæmoglobin under the influence of diminished pressure, has been observed by many other investigators, in human beings as well as in animals. Investigators are not united as to how this increase is brought about. The increase in the blood-corpuscles is not absolute, but is only relative, and it is considered by several observers that there is neither a new formation nor a dimin-

[1] Panum, Virchow's Arch., **29**; Worm Müller, l. c.

[2] Lacqueur, Deutsch. Arch. f. klin. Med., **110**; Cohnheim and Weber, *ibid.*, **110**.

ished destruction of the blood-corpuscles. A relative increase may be brought about in different ways. For example, another division of the blood-corpuscles in the vascular system has been supposed, whereby the blood-corpuscles accumulate in the capillaries, from which region the blood has been examined most often (ZUNTZ). It is also claimed that a concentration of the blood takes place by increased evaporation (GRAWITZ), and finally an increase in the blood-corpuscles has also been explained by assuming a contraction of the vascular system with the pressing out of plasma (BUNGE, ABDERHALDEN [1]). In connection with these experiments, it must be remarked that several trustworthy observations show that under the influence of diminished blood-pressure an actual increase in the red blood-corpuscles takes place. These and especially those of ZUNTZ and his co-workers have shown that under these conditions an increased activity occurs in the red bone-marrow. This question is still not clear. COHNHEIM and collaborators [2] have observed in man and dogs, that no essential increase in the blood-corpuscles and hæmoglobin occurs in high altitudes after 12 days. They do not dispute the action of a continued residence in high altitudes, and they also do not dispute such an action upon rabbits and mice. They explain this in these animals by a concentration of the blood due to a loss of water which is not replaced. In man and dogs on the contrary the loss of water brought about by perspiration is immediately replaced and the concentration of the blood prevented and the increase in the number of blood-corpuscles and of hæmoglobin is not observed.

A *decrease in the number of red corpuscles* occurs in anæmia from different causes. Every excessive hemorrhage causes an acute anæmia, or, more correctly, oligæmia. Even during the hemorrhage, the remaining blood becomes by diminished secretion and excretion, as also by an abundant absorption of parenchymous fluid, richer in water, somewhat poorer in proteins, and strikingly poorer in red blood-corpuscles. The oligæmia soon passes into an hydræmia. The amount of protein then gradually increases again; but the re-formation of the red blood-corpuscles is slower, and after the hydræmia follows also an oligocythæmia. After a little time the number of blood-corpuscles rises to normal. INAGAKI [3] has made thorough investigations on the changes which the number, volume and hæmoglobin content of the erythrocytes undergo after drawing blood as well as during regeneration. It is impossible here to enter more in

[1] The literature on this subject may be found in Abderhalden, Zeitschr. f. Biologie, **43**; van Voornveld, Pflüger's Arch., **92**.

[2] Höhenklima und Bergwanderungen, by N. Zuntz, A. Loewy, Franz Müller, and W. Caspari, Berlin, 1906; Otto Cohnheim, G. Kreglinger, L. Tobler, O. H. Weber, Zeitschr. f. physiol. Chem., 78 and Cohnheim, Ergebn. d. Physiologie, 1912, **12**.

[3] Zeitschr. f. Biol., **49**.

detail as to the results, but simply to state that they substantiate the previously known observation that, during regeneration, irregularties may occur in the relation between the quantity of hæmoglobin and the number of erythrocytes. A considerable decrease in the number of red corpuscles also occurs in chronic anæmia and chlorosis; still in such cases an essential decrease in the amount of hæmoglobin occurs without an essential decrease in the number of blood-corpuscles. The decrease in the amount of hæmoglobin is more characteristic of chlorosis than a decrease in the number of red corpuscles. The opinions on the changes in the blood in anæmia and chlorosis differ very considerably.[1]

A very considerable decrease in the number of red corpuscles (300,000–400,000 in 1 c.mm.) and diminution in the amount of hæmoglobin ($\frac{1}{3}$–$\frac{1}{10}$) occurs in pernicious anæmia (HAYEM, LAACHE, and others[2]). On the contrary, the individual red corpuscles are larger and richer in hæmoglobin than they ordinarily are, and the number stands in an inverse relation to the amount of hæmoglobin (HAYEM). Besides this the red corpuscles often, but not always, show in pernicious anæmia remarkable and extraordinary irregularities of form and size, which has been termed *poikilocytosis*.

The *number of leucocytes* may, as stated above, be increased under physiological conditions as well as after a meal rich in protein (physiological leucocytosis). Under pathological conditions a high leucocytosis may occur, and this is especially found in leucæmia, which is characterized by a very great abundance of leucocytes in the blood. The number of leucocytes is markedly increased in this disease, and indeed, not only absolutely, but also in relation to the number of red blood-corpuscles, which are diminished to a considerable extent in leucæmia. Leucæmic blood has a lower specific gravity than the ordinary blood (1035–1040), and a paler color, as if it were mixed with pus. The reaction is alkaline, but after death it is frequently acid, probably due to a decomposition of lecithin, which is often considerably increased in leucæmia. Volatile fatty acids, lactic acid, glycero-phosphoric acid, large amounts of purine bases, and so-called CHARCOT'S crystals (see Semen, Chapter XII) have also been found in leucæmic blood. The peptone (proteose) which is found in the leucæmic blood after death, and which does not exist in the fresh blood, is, according to ERBEN,[3] a digestive product which is

[1] Complete analyses of chlorotic blood may be found in Erben, Zeitschr. f. klin. Med., 47.

[2] Laache, Die Anämie (Christiania, 1883), which also contains the older literature. A complete chemical analysis of the blood has been made by Erben, Zeitschr. f. klin. Med., 40.

[3] Erben, Zeitschr. f. Heilkunde, 24, and Hofmeister's Beiträge, 5. See also Schumm, *ibid.*, 4 and 5. See also footnote 3, page 342.

produced by a tryptic enzyme which originates from the leucocytes as well as by traces of a peptic enzyme. A chemical analysis of leucæmic blood has been made by ERBEN.[1]

A great number of investigations have been made on the chemical composition of blood in disease. But as we have only a few analyses of the blood of healthy individuals, and as the possible variations under physiological conditions are little known, it is difficult to draw any positive conclusions from the analyses of pathological blood. Unfortunately, on account of the large number of contradictory deductions concerning the composition of the blood of diseased human beings, it is impossible to give a brief summary of the results, still the changes in the blood in disease must be of the greatest importance.

The *quantity of blood* is indeed somewhat variable in different species of animals and in different conditions of the body; in general we consider the entire quantity of blood in adults as about $\frac{1}{13}$–$\frac{1}{14}$ of the weight of the body, and in new-born infants about $\frac{1}{19}$. HALDANE and LORRAIN SMITH,[2] who have determined the quantity of blood by a new method, find in fourteen persons that it varies between $\frac{1}{16}$ and $\frac{1}{20}$ of the weight of the body. According to the same method OERUM[3] has determined the quantity of blood in men as about $\frac{1}{13}$ and in woman $\frac{1}{21}$ of the weight of the body. Fat individuals are relatively poorer in blood than lean ones. During inanition the quantity of blood decreases less quickly than the weight of the body (PANUM[4]), and it may therefore be also proportionally greater in starving individuals than in well-fed ones.

By careful bleeding, the quantity of blood may be considerably diminished without any dangerous symptoms. A loss of blood amounting to one-fourth of the normal quantity has as a sequence no lasting sinking of the blood-pressure in the arteries, because the smaller arteries accommodate themselves to the small quantities of blood by contracting (WORM MÜLLER[5]). A loss of blood amounting to one-third of the quantity reduces the blood-pressure considerably, and a loss of one-half of the blood in adults is dangerous to life. The more rapid the bleeding the more dangerous it is. New-born infants are very sensitive to loss of blood, and likewise fat, old, and weak persons cannot stand much loss of blood. Women can stand loss of blood better than men.

The quantity of blood may be considerably increased by the injection of blood from the same species of animal (PANUM, LANDOIS, WORM

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[1] Zeitschr. f. klin. Med., **66** (1908).
[2] Journ. of Physiol., **25**.
[3] Deutsch. Arch. f. klin. Med., **93** (1908).
[4] Virchow's Arch., **29**.
[5] Transfusion und Plethora, Christiania, 1875.

MÜLLER, PONFICK). According to WORM MÜLLER the normal quantity of blood may indeed be increased as much as 83 per cent without producing any abnormal conditions or lasting high blood-pressure. An increase of 150 per cent in the quantity of blood may, with a considerable variation in the blood-pressure, be directly dangerous to life (WORM MÜLLER). If the quantity of blood of an animal is increased by transfusion with blood of the same kind of animal, an abundant formation of lymph takes place. The water in excess is eliminated by the urine; and as the protein of the blood-serum is quickly decomposed, while the red blood-corpuscles are destroyed much more slowly (TSCHIRJEW, FORSTER, PANUM, WORM MÜLLER [1]), a polycythæmia is gradually produced.

The quantity of blood in the different organs depends essentially on their activity. During work the exchange of material in an organ is more pronounced than during rest, and the increased metabolism is connected with a more abundant flow of blood. Although the total quantity of blood in the body remains constant, the distribution of the blood in the various organs may be different at different times. As a rule the quantity of blood in an organ is an approximate measure of the more or less active metabolism going on in it, and from this point of view the distribution of the blood in the different organs is of interest. According to RANKE,[2] to whom we are especially indebted for our knowledge of the relation of the activity of the organs to the quantity of blood contained therein, of the total quantity of blood (in the rabbit) about one-fourth comes to the muscles in rest, one-fourth to the heart and the large blood-vessels, one-fourth to the liver, and one-fourth to the other organs.

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[1] Panum, Nord. med. Ark., **7**; Virchow's Arch., **62**; Landois, Centralbl. f. d. med. Wissensch., 1875, and Die Transfusion des Blutes, Leipzig, 1875; Worm Müller, Transfusion und Plethora; Ponfick, Virchow's Arch., **62**; Tschirjew, Arbeiten aus der physiol. Anstalt zu Leipzig, 1874, 292; Forster, Zeitschr. f. Biologie, **11**; Panum, Virchow's Arch., **29**.

[2] Die Blutvertheilung und der Thätigkeitswechsel des Organe, Leipzig, 1871.

CHAPTER VI.

CHYLE, LYMPH, TRANSUDATES AND EXUDATES.

I. CHYLE AND LYMPH.

THE lymph is at least in part the mediator in the exchange of constituents between the blood and the tissues. The bodies necessary for the nutrition of the tissues pass from the blood into the lymph, and the tissues deliver water, salts, and products of metabolism to the lymph. The lymph, therefore, originates partly from the blood and partly from the tissues. From a purely theoretical standpoint one can, according to HEIDENHAIN, differentiate between blood-lymph and tissue-lymph according to origin. It is impossible at the present time to separate completely that which comes from the one or the other source.

The lymph formed in the different organs and tissues has a different composition, and as the lymph is not obtained directly but only from the large lymph vessels, hence the lymph that we use for investigations is generally a mixture, whose composition may vary under certain conditions. The most easily obtained and best studied is the lymph from the thoracic duct. In starving individuals this lymph, which is called starvation lymph, does not essentially differ from other lymphs. After fatty food the lymph, which is called digestion lymph or *chyle*, differs from other lymphs by its great richness in very finely divided fat, which gives it a milky appearance, and which has led to the old name "lacteal fluid."

Chemically the lymph is the same as plasma, and contains, at least to a great extent, the same bodies. The observation of ASHER and BARBÈRA,[1] that the lymph contains poisonous metabolic products, does not contradict such an assumption, as no doubt these products are transferred to the blood with the lymph. Although the blood does not show the same poisonous action as the lymph, still this can be explained by the great dilution these bodies undergo in the blood, and the difference between blood-plasma and lymph is no doubt of a quantitative nature. This difference consists chiefly in that the lymph is poorer in proteins.

[1] Zeitschr. f. Biologie 36.

Lymph, like the plasma, contains *seralbumin*, *serglobulins*, *fibrinogen*, and *fibrin ferment*. The two last-mentioned bodies occur only in very small amounts; therefore the lymph coagulates slowly (but spontaneously) and yields but little fibrin. Like other liquids poor in fibrin ferment, lymph does not at once coagulate completely, but repeated coagulations take place.

The extractive bodies seem to be the same as in plasma. *Sugar* (or at least a reducing substance) is found in about the same quantity as in the blood-serum, namely, about 1 p. m. The *glycogen* detected by DASTRE[1] in the lymph occurs only in the leucocytes. According to RÖHMANN and BIAL, lymph contains a diastatic enzyme similar to that in blood-plasma, and LÉPINE[2] found that the chyle of a dog during digestion has great glycolytic activity. Lipases may also occur in lymph. The amount of *urea* has been determined by WURTZ[3] as 0.12–0.28 p. m. The *mineral bodies* appear to be the same as in plasma.

As form-elements, *leucocytes* and in certain cases *red blood-corpuscles* are common to both chyle and lymph. Chyle in fasting animals has the appearance of lymph. After fatty food it is, on the contrary, milky, due partly to small fat-globules, as in milk, and partly, indeed, mostly to finely divided fat. The nature of the *fat* occurring in chyle depends upon the kind of fat in the food. By far the greater part consists of neutral fat, and even after feeding with large quantities of free fatty acids, MUNK[4] found that the chyle contained chiefly neutral fat with only small amounts of fatty acids or soaps.

The *gases* of the entirely normal human lymph have not thus far been investigated. The gases from dog-lymph contain, according to HAMMARSTEN, only traces of oxygen, and consist of 37.4–53.1 per cent CO_2 and 1.6 per cent N, calculated at 0° C., and 760 mm. mercury. The chief mass of the carbon dioxide of the lymph seems to be in firm chemical combination. Comparative analyses of blood and lymph have shown that the lymph contains more carbon dioxide than arterial, but less than venous blood. The tension of the carbon dioxide of lymph is, according to PFLÜGER and STRASSBURG,[5] smaller than in venous, but greater than in arterial, blood.

The *quantitative composition of the chyle* must evidently be very variable. The specific gravity varies between 1.007 and 1.043. As an

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[1] Compt. rend. de soc. biol., **47**, and Compt. Rend., **120**; Arch. de Physiol. (5), **7**.

[2] Röhmann and Bial, Pflüger's Arch., **52**, **53**, and **55**; Lépine, Compt. Rend., **110**.

[3] Compt. Rend., **49**.

[4] Virchow's Arch., **80** and **123**. In regard to the analysis of the fat of chyle, see Erben, Zeitschr. f. physiol. Chem., **30**.

[5] Hammarsten, Die Gase der Hundelymphe, Arbeiten aus d. physiol. Anstalt zu Leipzig, 1871; Strassburg, Pflüger's Archiv, **6**.

example of the composition of human chyle two analyses will be given. The first is by OWEN-REES, of the chyle of an executed person, and the second by HOPPE-SEYLER,[1] of the chyle in a case of rupture of the thoracic duct. In the latter case the fibrin had previously separated. The results are in parts per 1000.

	No. 1.	No. 2.
Water	904.8	940.72 water
Solids	95.2	59.28 solids
Fibrin	Traces	.....
Albumin	70.8	36.67 albumin
Fat	9.2	7.23 fat
Remaining organic bodies	10.8	2.35 soaps 0.83 lecithin 1.32 cholesterin 3.63 alcohol extractives 0.58 water extractives
Salts	4.4	6.80 soluble salts 0.35 insoluble salts

The quantity of fat is very variable and may be considerably increased by partaking of food rich in fats. I. MUNK and A. ROSENSTEIN [2] have investigated the lymph or chyle obtained from a lymph fistula at the end of the upper third of the leg of a girl eighteen years old and weighing 60 kg., and the highest quantity of fat in the chylous lymph was 47 p. m. after partaking of fat. In the starvation lymph from the same patient they found only 0.6–2.6 p. m. fat. The quantity of soaps was always small, and on partaking of 41 grams of fat the quantity of soaps was only about $\frac{1}{10}$ of the neutral fats. SCHUMM [3] found in the creamy contents of a chylous cyst of the mesentery, 357.8 p. m. fat and comparatively large amounts of calcium soaps.

A great many analyses of chyle from animals have been made, and they chiefly show the fact that the chyle is a liquid with a very changeable composition which stands closely related to blood-plasma, but with the principal difference that it contains more fat and less solids. The reader is referred to special works for these analyses, as, for example, to v. GORUP-BESANEZ'S "Lehrbuch der physiologischen Chemie," 4th edition.

The *composition of the lymph* is also very changeable, and its specific gravity shows about the same variation as the chyle. In the following analyses, 1 and 2, made by GUBLER and QUEVENNE, are the results obtained from lymph of the upper part of the thigh of a woman aged thirty-nine; and 3, made by v. SCHERER, is an analysis of lymph from

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[1] Owen-Rees, cited from Hoppe-Seyler's Physiol. Chem., 595; Hoppe-Seyler, *ibid.*, 597. See also Carlier, Brit. Med. Journ., 1902, 175, and T. Sollmann, Amer. Journ. of Physiol., 17.

[2] Virchow's Arch., 128.

[3] Zeitschr. f. physiol. Chem., 49.

the sac-like dilated lymphatic vessels of the spermatic cord. No. 4 was made by C. SCHMIDT [1] the data being obtained from lymph from the neck of a colt. The results are expressed in parts per 1000.

	1	2	3	4
Water	939.9	934.8	957.6	955.4
Solids	60.1	65.2	42.4	44.6
Fibrin	0.5	0.6	0.4	2.2
Albumin	42.7	42.8	34.7	.....
Fat, cholesterin, lecithin	3.8	9.2	.....	35.0
Extractive bodies	5.7	4.4	.....	.....
Salts	7.3	8.2	7.2	7.5

The salts found by C. SCHMIDT in the lymph of the horse have the following composition, calculated in parts per 1000 parts of the lymph:

Sodium chloride	5.67
Soda	1.27
Potash	0.16
Sulphuric acid	0.09
Phosphoric acid united with alkalies	0.02
Earthy phosphates	0.26

In the cases investigated by MUNK and ROSENSTEIN the quantity of solids in the fasting condition varied between 35.7 and 57.2 p. m. This variation was essentially dependent upon the extent of secretion, so that the low amount coincides with a more active secretion, and the reverse in the other case. The chief portion of the solids consisted of proteins, and the relation between globulin and albumin was as 1:2.4 to 4. The mineral bodies in 1000 parts lymph (chylous) were: NaCl 5.83; Na_2CO_3 2.17; K_2HPO_4 0.28; $Ca_3(PO_4)_2$ 0.28; $Mg_3(PO_4)_2$ 0.09; and $Fe(PO_4)$0.025. The quantity of titratable alkali in the lymph is much smaller than in the blood. CARLSON, GREER and LUCKHARDT[2] have recently made comparative estimations of NaCl in blood-serum and lymph of the same individual (horse and dog) and find that the lymph is regularly richer in chlorides, a condition which, according to them, is difficult to reconcile with the view of the filtration and transudation processes in the formation of lymph.

In this connection it must be recalled that according to many investigators the lymph has a somewhat higher osmotic pressure and therefore a somewhat greater molecular concentration than the serum. CARLSON, GREER and BECHT[3] found, nevertheless, that the osmotic pressure of neck-lymph in the dog is often lower than the serum.

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[1] Gubler and Quevenne, cited from Hoppe-Seyler's Physiol. Chem., 591; v. Scherer, *ibid.*, 591; C. Schmidt, *ibid.*, 592. See also Zaribnicky, Zeitschr. f. physiol. Chem., 78.

[2] Amer. Journ. of Physiol., **22** (1908).

[3] *Ibid.*, **19** (1907).

Under special conditions the lymph may be so rich in finely divided fat that it appears like chyle. Such lymph has been investigated by HENSEN in a case of lymph fistula in a ten-year-old boy, and by LANG [1] in a case of lymph fistula in the upper part of the left thigh of a girl of seventeen. The lymph investigated by HENSEN varied in the quantity of fat, as an average of nineteen analyses, between 2.8 and 36.9 p. m.; while that investigated by LANG contained 24.85 p. m. of fat.

The quantity of lymph secreted must naturally change considerably under various conditions, and there are no means of measuring it. The size of the flow of lymph is, as HEIDENHAIN suggests, no measure of the abundance of supply of nutritive material to the organs, and the lymph-tubes act according to him as "drain-tubes," removing the excess of fluid from the lymph fissures as soon as the pressure therein rises to a certain height. Attempts have been made to determine the quantity of lymph flowing in 24 hours through the thoracic duct of animals. According to HEIDENHAIN the quantity averages 640 cc. for a dog weighing 10 kilos.

Determinations of the quantity of lymph in man have also been attempted. NOËL-PATON [2] obtained 1 cc. of lymph per minute from the severed thoracic duct of a patient weighing 60 kilos. The quantity in the 24 hours cannot be calculated from this amount. In the case of MUNK and ROSENSTEIN, 1134–1372 grams of chyle were collected within 12–13 hours after partaking of food. In the fasting condition or after starving for 18 hours they found 50 to 70 grams per hour, sometimes 120 grams and above, especially in the first few hours after powerful muscular exercise.

Several circumstances have a marked influence on the extent of lymph secretion. During starvation less lymph is secreted than after partaking of food. NASSE [3] has observed that the formation of lymph in dogs is increased 36 per cent more after feeding with meat than after feeding with potatoes, and about 54 per cent more than after 24 hours' deprivation of food. In this connection mention must be made of the important observations of ASHER and BARBÈRA [4] that with pure protein diet the lymph current is increased in the thoracic cavity, and also that the increase in the lymph secretion runs parallel with the elimination of nitrogen in the urine, i.e., with the absorption of the protein from the digestive tract.

An increase in the total quantity of blood, as by transfusion of blood, also especially in preventing the flow of blood by means of ligatures,

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[1] Hensen, Pflüger's Arch., 110; Lang, see Maly's Jahresber., 4.

[2] Journ. of Physiol., 11.

[3] Cited from Hoppe-Seyler, Physiol. Chem., 593.

[4] The works of Asher and collaborators, Barbèra, Gies, and Busch, upon lymph formation may be found in Zeitschr. f. Biologie, 36, 37, 40.

causes an increase in the quantity of lymph. According to HEIDENHAIN, on the contrary, a very considerable change in the pressure in the aorta causes only a little change in the abundance of the lymph flow. The quantity of lymph may be raised by powerfully active and passive movements of the limbs (LESSER). Under the influence of curare, an increase of the lymph secretion is observed (PASCHUTIN, LESSER [1]), and the quantity of solids in the lymph is also increased.

The bodies inciting lymph flow, the so-called *lymphagogues*, are of especially great interest, and they may, according to HEIDENHAIN,[2] be divided into two different chief groups. The lymphagogues of the first series—extracts of crab-muscles, blood-leech, anodons, liver and intestine of dogs, as well as peptone and egg albumin, strawberry extracts, metabolic products of bacteria and others—cause a greatly increased secretion of lymph without raising the blood-pressure, and in this way the blood-plasma becomes poorer in proteins and the lymph richer than before. For the formation of this lymph, which HEIDENHAIN designates blood-lymph, we must admit with him that a special secretory activity of the capillary-wall endothelium exists. The lymphagogues of the second series, such as sugar, urea, sodium chloride, and other salts, also cause an abundant lymph formation. The blood, as well as the lymph, thereby becomes richer in water. This increased amount of water depends, according to HEIDENHAIN, upon an increased delivery of water by the tissue-elements, and this lymph is chiefly tissue-lymph, in his opinion. Diffusion is no doubt of great importance in the formation of this lymph, but the secretory activity of the endothelium is also of importance, at least for certain bodies, such as sugar.

In the past, the formation of lymph was explained in a purely physical way by the united action of filtration from the blood and the osmosis between the blood and tissue-fluid. Later HEIDENHAIN and also HAMBURGER ascribed a special activity to the capillary endothelium, assuming that they take part in the formation of lymph in a secretory manner. The above-mentioned observations on the greater NaCl content in the lymph as compared to the plasma as well as the regularly found higher osmotic pressure of the lymph speak for such a view.

According to ASHER and his collaborators (BARBÈRA, GIES and BUSCH) the lymph is a product of the work of the organs. Its amount is dependent upon an increased or diminished activity of the organs,

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[1] Lesser, Arbeiten aus der physiol. Anstalt zu Leipzig, Jahrgang, 6; Paschutin, *ibid.*, 7.

[2] Heidenhain, Pflüger's Arch., 49; Hamburger, Zeitschr. f. Biologie, 27 and 30. See especially Ziegler's Beitr. zur Path. u. zur allg. Pathol., 14, 443; also Arch. f. (Anat. u.) Physiol., 1895 and 1896.

and the lymph is therefore a measure of the work in these. The close relation between lymph formation and the work of organs has also been shown for several of them, especially for the liver. STARLING has shown that after the introduction of lymphagogues of the first series, chiefly liver lymph is secreted, which he claims is a proof against HEIDENHAIN'S view, and he explains the increased permeability of the vessel wall by the fact that these bodies have an irritating, poisonous action. On the contrary, ASHER explains this increased lymph flow by the statement that the substance in question—as well as those influences which incite the activity of the liver—produces an increased formation of lymph in these organs. This view is supported by experiments upon the action of lymphagogues on blood coagulation and liver activity (DELEZENNE and others), for, according to GLEY, these bodies have at the same time a lymphagogue action and an action upon the secretion of the glands. We have no direct evidence of the action of the lymphagogues of the first series upon the organs, but we know from KUSMINE'S work that peptone, leech extract, and the extractives of the crab-muscles act directly upon the liver-cells and bring about morphological changes. The connection between organ activity and lymph formation has also been shown upon muscles and glands by others besides the above-mentioned investigators (HAMBURGER, BAINBRIDGE [1]).

The extent of organ work essentially influences the quantity and properties of the lymph. Still from this we cannot draw any positive conclusions as to whether the lymph formation is brought about by physico-chemical processes alone or whether in this process a specific, not closely definable secretory force is at work at the same time. In regard to this much-disputed question, attention must be called in the first place to the fact that the important works of HEIDENHAIN, HAMBURGER, LAZARUS-BARLOW, and others, as well as the investigations of ASHER and GIES and of MENDEL and HOOKER [2] upon the lengthy post-mortem lymph flow, have shown that the older filtration hypothesis is untenable.

That osmotic processes play an important rôle in the lymph formation is generally admitted and that the work of the glands and tissue cells must cause a difference in the osmotic pressure on both sides of the capillary walls, has been shown by the researches of many investigators (KORÁNYI, STARLING, ROTH, ASHER and others). That this is so follows from several circumstances, and especially from the fact that, in disassimila-

[1] In regard to the works cited, as well as the literature upon lymph formation, see Ellinger, "Die Bildung der Lymphe," Ergebnisse der Physiol., I, Abt. 1, 1902, and Asher, Biochem. Centralbl., 4.

[2] Amer. Journ. of Physiol., 7. See also footnote 1.

tion in the cells, bodies of high molecular weight are split into a number of smaller molecules, which latter, either directly, if they leave the cells and pass into the tissue-fluid, or indirectly, when they remain in the cells, produce an increase in the osmotic tension within the cells, and in this way cause a taking up of water from the fluid, and must therefore increase the osmotic pressure of the tissue-fluids. As the cells can by synthesis build up highly complex constituents from simple molecules, and as the chief products of catabolism are carbon dioxide and water, it is difficult to explain these intricate conditions. Still, irrespective of whatever view, a change in one or the other direction in the osmotic pressure upon both sides of the capillary wall must be produced thereby. Whether this and other physico-chemical processes are alone sufficient to explain the lymph formation (COHNSTEIN, ELLINGER) remains an open and disputed question.[1]

II. TRANSUDATES AND EXUDATES.

The serous membranes are normally kept moistened by liquids whose quantity is sufficient only in a few instances, as in the pericardial cavity and the subarachnoidal space, for a complete chemical analysis to be made of them. Under diseased conditions an abundant transudation may take place from the blood into the serous cavities, into the subcutaneous tissues, or under the epidermis; and in this way pathological transudates are formed. Such true transudates, which are similar to lymph, are generally poor in form-elements and leucocytes, and yield only very little or almost no fibrin, while the inflammatory transudates, the so-called exudates, are generally rich in leucocytes and yield proportionally more fibrin. As a rule, the richer a transudate is in leucocytes the closer it stands to pus, while a diminished quantity of leucocytes renders it more nearly like a real transudate or lymph.

It is ordinarily accepted that filtration is of the greatest importance in the formation of transudates and exudates. The facts coincide with this view that all these fluids contain the salts and extractive bodies occurring in the blood-plasma in about the same quantity as the blood-plasma, while the amount of proteins is habitually smaller. While the different fluids belonging to this group have about the same quantities of salts and extractive bodies, they differ from one another chiefly in containing differing quantities of protein and form-elements, as well as varying quantities of transformation and decomposition products of these latter—changed blood-coloring matters, cholesterin, etc. The

[1] On this question see Ellinger, "Die Bildung der Lymphe," Ergebnisse der Physiologie, I, Abt. 1, 355, and Asher, Biochem. Centralbl., 4, pp. 1 and 45.

correspondence in the amount of salts and extractive bodies present in the blood and in transudates supplies just as little proof for a filtration as it does for the formation of lymph; but still it cannot be doubted for other reasons that filtration is often of great importance in the formation of a transudate. To what extent filtration is active in the perfectly normal vascular wall cannot be answered.

The altered permeability of the capillary walls in disease is a second important factor in the formation of transudates. The circumstance that the greatest quantity of protein occurs in transudates in inflammatory processes, to which is also due the abundant quantity of form-elements in such transudates, has been explained by this hypothesis. The greater quantity of protein in the transudates in formative irritation is in great part explained by the large amount of destroyed form-elements. The interesting observation made by PAIJKULL,[1] that in those cases in which an inflammatory irritation has taken place the fluid contains nucleoalbumin (or nucleoprotein?), while this substance does not occur in transudates in the absence of inflammatory processes, can be explained by the presence of form-elements. Still, such a phosphorized protein substance does not occur in all inflammatory exudates.

As the secretory importance of the capillary endothelium has been made probable by the investigations of HEIDENHAIN, it is *a priori* to be expected that an abnormally increased secretory activity of the endothelium is a cause of transudates. Those observations which substantiate such an assumption can also be explained just as well by assuming a changed permeability of the capillary walls.

The varying quantities of protein observed by C. SCHMIDT[2] in the tissue-fluids in different vascular regions can perhaps be explained by the different condition of the capillary endothelium. For example, the amount of protein in the PERICARDIAL, PLEURAL, and PERITONEAL FLUIDS is considerably greater than in those fluids which are found in the SUBARACHNOIDAL SPACE, in the SUBCUTANEOUS TISSUES, or in the AQUEOUS HUMOR, which are poor in protein. The condition of the blood also greatly affects the transudates, for in hydræmia the amount of protein in the transudate is very small. With the increase in the age of a transudate, of a hydrocele fluid for instance, the quantity of protein is increased, probably by resorption of water, and indeed exceptional cases may occur in which the amount of protein, without any previous hemorrhage, is even greater than in the blood-serum.

The proteins of transudates are chiefly seralbumin, serglobulin, and a little fibrinogen. Proteoses and peptones do not occur, excepting

[1] See Maly's Jahresber., 22.

[2] Cited from Hoppe-Seyler, Physiol. Chem., 607.

perhaps in the cerebrospinal fluid, and in those cases where an autolysis has taken place in the liquid.[1] The non-inflammatory transudates do not as a rule undergo spontaneous coagulation or do so only very slowly. On the addition of blood or blood-serum they coagulate. Inflammatory exudates coagulate spontaneously, and PAIJKULL has shown that these often contain nucleoprotein (or nucleoalbumin). In inflammatory exudates a protein substance has been habitually observed which is precipitated by acetic acid, but which does not occur in transudates, or only in very small quantities. This substance, which has been observed and studied by MORITZ, STAEHELIN, UMBER, and RIVALTA, is claimed by the first three observers to be free from phosphorus, while RIVALTA considers it to be a phosphorized pseudoglobulin. UMBER calls it *serosamucin*, although it yields only very little reducing carbohydrate. According to JOACHIM[2] it is only a part of the globulin, a view which cannot be correct for all cases. v. HOLST[3] has so far substantiated UMBER'S observation in that he has isolated a mucin substance from an ascitic fluid in carcinoma of the stomach and the peritoneum, which seemed to be identical with UMBER'S serosamucin, as well as with the synovial mucin. There does not seem to be any doubt that in transudates and exudates, different protein substances may occur under different circumstances, although the globulins form besides seralbumin the principal mass of the protein bodies. Mucoid substances, which were first observed by HAMMARSTEN in certain cases of ascites without complications with ovarial tumors, and which are cleavage products of a more complicated substance, seem according to PAIJKULL[4] to be regular constituents of transudates and are closely related to the above-mentioned serosamucin. The occurrence of the above-mentioned substances precipitable by acetic acid, the globulins (RIVALTA) and the nucleoproteins, in puncture fluids, has been recognized as of very great importance in the differential diagnosis between transudates and exudates.

There are numerous investigations on the relation between globulin and seralbumin, and JOACHIM has determined the relation between euglobulin and the total globulin. No conclusive results can be drawn from these determinations. The relation between globulin and seralbumin varies very much in different cases, but, as HOFFMANN and

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[1] Umber, Münch. med. Wochenschr., 1902, and Berlin. klin. Wochenschr., 1903. In regard to the autolysis in transudates, see also Galdi, Biochem. Centralbl., **3**; Eppinger, Zeitschr. f. Heilkunde, **25**, and Zak, Wien. klin. Wochenschr., 1905.

[2] Paijkull, l. c.; Moritz, Münch. med. Wochenschr., 1903; Staehelin, *ibid.*, 1902, Umber, Zeitschr. f. klin. Med., 48; Rivalta, Biochem. Centralbl., **2** and **5**; Joachim; Pflüger's Arch., **93**.

[3] Zeitschr. f. physiol. Chem., **43**.

[4] Hammarsten, *ibid.*, **15**; Paijkull, l. c.

PIGEAND [1] have shown, the variation is in each case the same as in the blood-serum of the individual.

The specific gravity runs almost parallel with the quantity of protein. The varying specific gravity has been suggested as a means of differentiation between transudates and exudates by REUSS,[2] as the first often show a specific gravity below 1015–1010, while the others have a specific gravity of 1018 or above. This rule holds good in many, but not in all cases.

The *gases* of the transudates consist of carbon dioxide besides small amounts of nitrogen and traces of oxygen. The tension of the carbon dioxide is greater in the transudates than in the blood. When mixed with pus, the amount of carbon dioxide is decreased.

The *extractives* are, as above stated, the same as in the blood-plasma. *Urea* seems to occur in very variable amounts. *Sugar* also occurs in transudates, but it is not known to what extent the reducing power is due to other bodies, as in blood-serum. A reducing, non-fermentable substance has been found by PICKARDT in transudates. The sugar is generally glucose, but fructose seems to have been found [3] in several cases. *Sarcolactic acid* has been found by C. KÜLZ in the pericardial fluid from oxen. *Succinic acid* has been found in a few cases in hydrocele fluids, while in other cases it is entirely absent. *Leucine* and *tyrosine* have been found in transudates from diseased livers and pus-like transudates which have undergone decomposition, and after autolysis. Among other extractives found in transudates must be mentioned *allantoin* (MOSCATELLI [4]), *uric acid*, *purine bases*, *creatine*, *inosite*, and *pyrocatechin* (?).

The division of the nitrogenous substances in human transudates and exudates has so far been little studied. OTORI found that no essential difference exists between serous exudates and transudates in regard to the quantity of urea and amino-acids. The amount of total nitrogen and proteins runs parallel with the specific gravity, and the same is generally true for the absolute values for ammonia nitrogen and purine nitrogen. According to the investigations of CZERNECKI,[5] in pathological puncture fluids, also *oxyproteic acids* (see Chapter XIV on the urine) occur and which represent 13.3—25.9 per cent of the total nitrogen of the protein free filtrate. The question as to the amount of

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[1] Joachim, l. c.; Hoffmann, Arch. f. exp. Path. u. Pharm., **16**; Pigeand, see Maly's Jahresber., **16**.

[2] Reuss, Deutsch. Arch. f. klin. Med., **28**. See also Otto, Zeitschr. f. Heilkunde, **17**.

[3] Pickardt, Berl. klin. Wochenschr., 1897. See also Rotmann, Münch. med. Wochenschr., 1898; Neuberg and Strauss, Zeitschr. f. physiol. Chem., **36**; Sittig, Bioch. Zeitschr. **21**.

[4] C. Külz, Zeitschr. f. Biologie, **32**; Moscatelli, Zeitschr. f. physiol. Chem. **13**.

[5] Otori, Zeitschr. f. Heilk. **25**; Czernecki, Maly's Jahresb., **29**.

urea nitrogen and amino-acid nitrogen in such fluids must, under these circumstances, require further study.

The investigations upon the molecular concentration have shown that no essential and constant difference exists between exudates and transudates. The osmotic concentration and the concentration of the electrolytes are as a rule the same as in blood-serum, although sometimes rather divergent results have been found. The concentration of the electrolytes shows, according to BODON,[1] like the blood-serum, much less variation than the total concentration. The alkalinity determined by titration is about the same in transudates and exudates, and is equal to that of the blood-serum. The determination of the HO ion concentration has shown that the transudates and exudates in this regard are about as neutral as the blood-serum (BODON).

As above stated, irrespective of the varying number of form-elements contained in the different transudates, the quantity of protein is the most characteristic chemical distinction in the composition of the various transudates; therefore a quantitative analysis is of importance only in so far as it considers the quantity of protein. On this account, in the following, relative to the quantitative composition, stress will be put on the quantity of protein.

Pericardial Fluid. The quantity of this fluid is, even under physiological conditions, so large that a sufficient quantity for chemical investigation has been obtained (from persons who had been executed). This fluid is lemon-yellow in color, somewhat sticky, and yields more *fibrin* than other transudates. The amount of solids, according to the analyses performed by v. GORUP-BESANEZ, WACHSMUTH, and HOPPE-SEYLER,[2] is 37.5–44.9 p. m., and the amount of protein is 22.8-24.7 p. m. The analysis made by HAMMARSTEN of a fresh pericardial fluid from a young man who had been executed yielded the following results, calculated in 1000 parts by weight.

Water	960.85		
Solids	39.15		
Proteins	28.60	Fibrin	0.31
		Globulin	5.95
		Albumin	22.34
Soluble salts	8.60	NaCl	7.28
Insoluble salts	0.15		
Extractive bodies	2.00		

FRIEND[3] found almost the same composition for a pericardial fluid from a horse, with the exception that this liquid was relatively richer

[1] Pflüger's Arch., **104**, where literature on this subject may be found.

[2] v. Gorup-Besanez, Lehrbuch d. physiol Chem., 4. Aufl., 401; Wachsmuth, Virchow's Arch., **7**; Hoppe-Seyler, Physiol. Chem., 605.

[3] Halliburton, Text-book of Chem. Physiol., etc., London, 1891.

in globulin. The ordinary statement that pericardial fluids are richer in fibrinogen than other transudates is hardly based on sufficient proof. In a case of chylopericardium, which was probably due to the rupture of a chylous vessel, or caused by a capillary exudation of chyle because of stoppage, HASEBROEK [1] found in 1000 parts of the fluid 103.61 parts solids, 73.79 parts proteins, 10.77 parts fat, 3.34 parts cholesterin, 1.77 parts lecithin, and 9.34 parts salts.

The **pleural fluid** occurs under physiological conditions in such small quantities that a chemical analysis of it cannot be made. Under pathological conditions this fluid may show very variable properties. In certain cases it is nearly serous, in others again sero-fibrinous, and in others similar to pus. There is a corresponding variation in the specific gravity and the properties in general. If a pus-like exudate is kept enclosed for a long time in the pleural cavity, a more or less complete maceration and solution of the pus-corpuscles is found to take place. The ejected yellowish-brown or greenish fluid may then be as rich in solids as the blood-serum; and an abundant flocculent precipitate of a nucleoalbumin or nuceloprotein (the *pyrin* of early writers) may be obtained on the addition of acetic acid. This precipitate is soluble with difficulty in an excess of acetic acid.

Numerous analyses, by many investigators,[2] of the quantitative composition of pleural fluids under pathological conditions have been published. From these analyses we learn that in hydrothorax the specific gravity is lower and the quantity of protein less than in pleuritis. In the first case the specific gravity is generally less than 1.015, and the quantity of protein 10–30 p. m. In acute pleuritis the specific gravity is generally higher than 1.020, and the quantity of protein 30–65 p. m. The quantity of fibrinogen, which in hydrothorax is about 0.1 p. m., may amount to more than 1 p. m. in pleuritis. In pleurisy with an abundant accumulation of pus, the specific gravity may rise even to 1.030 according to the observations of HAMMARSTEN. The quantity of solids is often 60–70 p. m., and may be even more than 90–100 p. m. (HAMMARSTEN). Mucoid substances have also been detected in pleural fluids by PAIJKULL. Cases of chylous pleurisy are also known; in such a case MÉHU [3] found 17.93 p. m. fat and cholesterin in the fluid.

The quantity of **peritoneal fluid** is very small under physiological conditions. The investigations refer only to the fluid under diseased

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[1] Zeitschr. f. physiol. Chem., **12**.

[2] See the works of Méhu, Runeberg, F. Hoffmann, Reuss, all of which are cited in Bernheim's paper in Virchow's Arch., **131**, 274. See also Paijkull, l. c., and Halliburton's Text-book, 346; Joachim, l. c.

[3] Arch. gén. de méd., 1886, **2**, cited from Maly's Jahresber., **16**.

conditions (*ascitic fluid*). The color, transparency, and consistency of these may vary greatly.

In cachectic conditions or a hydræmic condition of the blood the fluid has little color, is milky, opalescent, watery, does not coagulate spontaneously, has a very low specific gravity, 1.006–1.010–1.015, and is almost free from form-elements. The ascitic fluid in portal stagnation, or in general venous congestion, has a low specific gravity and contains ordinarily less than 20 p. m. protein, although in certain cases the quantity of protein may rise to 35 p. m. In carcinomatous peritonitis it may have a cloudy, dirty-gray appearance, due to its richness in form-elements of various kinds. The specific gravity is then higher, the quantity of solids greater, and it often coagulates spontaneously. In inflammatory processes it is straw- or lemon-yellow in color, somewhat cloudy or reddish, due to leucocytes and red blood-corpuscles, and from great richness in leucocytes it may appear more like pus. It coagulates spontaneously and may be relatively richer in solids. It contains regularly 30 p. m. or more protein (although exceptions with less protein occur), and may have a specific gravity of 1.030 or above. On account of the rupture of a chylous vessel, the ascitic fluid may be rich in very finely emulsified fat (CHYLOUS ASCITES). In such cases 3.86–10.30 p. m. fat has been found in the ascitic fluid (GUINOCHET, HAY [1]), and even 17–43 p. m. has been found by MINKOWSKI.

As first shown by GROSS, an ascitic fluid may have a chylous appearance without the presence of fat, i.e., pseudochylous. The cause of the chylous properties of a transudate is not known, although numerous investigators, such as GROSS, BERNERT, MOSSE, and STRAUSS, have studied the subject; several observations, however, seem to show that it is connected with the amount of lecithin contained therein. In a case investigated by H. WOLFF [2] the oleic-acid ester of cholesterin was combined either chemically or molecularly with the euglobulin.

By admixture of ascitic fluid with that from an ovarian cyst the former may sometimes contain pseudomucin (see Chapter XII). There are also cases in which the ascitic fluid contains mucoids which may be precipitated by alcohol after removal of the proteins by coagulation at boiling temperature. Such mucoids, which yield a reducing substance on boiling with acids, have been found by HAMMARSTEN in tuberculous peritonitis and in cirrhosis hepatis syphilitica in men. According to the investigations of PALJKULL, these substances seem to occur often and perhaps habitually in the ascitic fluids.

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[1] Guinochet, see Strauss, Arch. de Physiol., 18. See Maly's Jahresber., 16, 475.

[2] Gross, Arch. f. exp. Path. u. Pharm., 44; Bernert, *ibid.*, 49; Mosse, Leyden's Festschrift, 1901; Strauss, cited in Biochem. Centralbl., 1, 437; Wolff, Hofmeister's Beiträge, 5.

As the quantity of protein in ascitic fluids is dependent upon the same factors as in other transudates and exudates, it is sufficient to give the following example of the composition, taken from BERNHEIM'S [1] treatise. The results are expressed in 1000 parts of the fluid:

	Max.	Min.	Mean.
Cirrhosis of the liver	34.5	5.6	9.69—21.06
Bright's disease	16.11	10.10	5.6 —10.36
Tuberculous and idiopathic peritonitis	55.8	18.72	30.7 —37.95
Carcinomatous peritonitis	54.20	27.00	35.1 —58.96

JOACHIM found the highest relative globulin amounts and lowest albumin percentages in cirrhosis; in carcinoma, on the contrary, the lowest globulin and the highest albumin. The values in cardiac stagnation stand between the cirrhosis and carcinoma percentages.

Urea has also been found in ascitic fluids, sometimes only as traces, sometimes in larger quantities (4 p. m. in albuminuria), also *uric acid*, *allantoin* in cirrhosis of the liver (MOSCATELLI), *xanthine*, *creatine*, *cholesterin*, *sugar*, *diastatic* and *proteolytic enzymes*, and according to HAMBURGER [2] also a *lipase*.

Hydrocele and Spermatocele Fluids. These fluids differ essentially from each other in various ways. The hydrocele fluids are generally colored light or dark yellow, sometimes brownish with a shade of green. They have a relatively higher specific gravity, 1.016–1.026, with a variable but generally higher amount of solids, an average of 60 p. m. They sometimes coagulate spontaneously, sometimes only after the addition of fibrin ferment or blood. They contain *leucocytes* as chief form-elements. Sometimes they contain smaller or larger amounts of *cholesterin crystals*.

The spermatocele fluids, on the contrary, are as a rule colorless, thin, and cloudy like water mixed with milk. They sometimes have an acid reaction. They have a lower specific gravity, 1.006–1.010, a lower amount of solids—an average of about 13 p. m.—and do not coagulate either spontaneously or after the addition of blood. They are, as a rule, poor in protein and contain *spermatozoa*, *cell-detritus*, and *fat-globules* as form constituents. To show the unequal composition of these two kinds of fluids we will give the average results (calculated in parts per 1000 parts of the fluid) of seventeen analyses of hydrocele fluids and four of spermatocele fluids made by HAMMARSTEN.[3]

	Hydrocele.	Spermatocele.
Water	938.85	986.83
Solids	61.15	12.17
Fibrin	0.59	.....
Globulin	13.25	0.59
Seralbumin	35.94	1.82
Ether extractive bodies	4.02	
Soluble salts	8.60	10.76
Insoluble salts	0.66	

[1] l. c. As it was impossible to derive mean figures from those given by Bernheim, the author has given the maximum and minimum of the averages given by him.

[2] Arch. f. (Anat. u.) Physiol., 1900, 433.

[3] Upsala Läkaref. Förh., 14, and Maly's Jahresber., 8, 347.

In the hydrocele fluid traces of *urea* and a reducing substance have been found, and in a few cases also *succinic acid* and *inosite*. A hydrocele fluid may, according to DEVILLARD,[1] sometimes contain paralbumin or metalbumin (?). Cases of chylous hydrocele are also known.

Cerebrospinal Fluid. The cerebrospinal fluid is thin, water-clear, of low specific gravity, 1.007–1.008. The spina bifida fluid is very poor in solids, 8–10 p. m. with only 0.19–1.6 p. m. protein. The fluid of chronic hydrocephalus is somewhat richer in solids (13–19 p. m.) and proteins. The amount of protein in the cerebrospinal fluid seems to be rather variable under diseased conditions and FRENKEL-HEIDEN[2] found 0.875–3 p. m. protein in the lumbar fluid in progressive paralysis and 0.7–2.8 p. m. protein in tuberculous meningitis. In the perfectly fresh fluid from healthy calves NAWRATZKI found an average of 0.22 p. m. protein.

According to HALLIBURTON the protein of the cerebrospinal fluid is a mixture of *globulin* and *proteose;* occasionally some peptone occurs, and more rarely, in special cases, seralbumin appears. The conclusions of HALLIBURTON on the occurrence of *proteose* do not coincide with the observations of other investigators (PANZER, SALKOWSKI[3]). In general paralysis, HALLIBURTON and MOTT obtained a *nucleoprotein* in the cerebrospinal fluid. *Choline* occurs in several diseases, as in general paralysis, brain-tumors, tabes dorsalis, and epilepsy (HALLIBURTON and MOTT, DONATH, ROSENHEIM). According to KAUFMANN[4] we are not here dealing with choline but with another base. *Glucose*, or at least a fermentable sugar, occurs habitually in the cerebrospinal fluid, while the claims of HALLIBURTON as to the occurrence of a substance similar to pyrocatechin could not be substantiated in calves and men by NAWRATZKI,[5] and hence this substance does not exist in all cerebrospinal fluids. *Urea* occurs in cerebrospinal fluids, but not always. In the cases investigated by FRENKEL-HEIDEN indeed all the rest-nitrogen occurred as urea and the urea-nitrogen varied in different pathological cases between 0.196–1.12 p. m. *Lactic Acid* has been found by LEHNDORFF and BAUMGARTEN[6] in many pathological cases. The quantity of NaCl is regularly much greater than the KCl, 6–7 p. m. NaCl against

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[1] Bull. Soc. chim., **42**, 617.

[2] Bioch. Zeitschr., **2**.

[3] Halliburton's Text-book; Panzer, Wein. klin. Wochenschr., 1899; Salkowski, Jaffé Festschrift, 265.

[4] Halliburton and Mott, Phil. Transact. Roy. Soc. London, Series B, **191**; Donath, Zeitschr. f. physiol Chem., **39** and **42**; see also Mansfield, *ibid.*, **42**; Rosenheim, Journ. of Physiol., **35**; Kaufmann, Zeitschr. f. physiol. Chem., **66**.

[5] Zeitschr. f. physiol. Chem., **23**. See also Rossi, *ibid.*, **39** (literature).

[6] Zeitschr. f. exp. Path. u. Therap., **4** (literature).

about 0.4 p. m. KCl, and the variable relation between potassium and sodium is probably due, according to SALKOWSKI,[1] to the absence or presence of fever during the formation of the exudate; the amount of potassium is high in the acute cases and low in the chronic ones. According to LANDAU and HALPERN [2] a certain antagonism seems to exist between nitrogen and sodium chloride, as the highest results of the first correspond to the lowest results of the other. According to CAVAZZANI,[3] who has especially studied the cerebrospinal fluids, the alkalinity of these fluids is considerably less than that of the blood and independent of this last fluid. For this and several other reasons CAVAZZANI draws the conclusion that the cerebrospinal fluid is formed by a true secretory process.

A large number of investigations on the cerebrospinal fluid have been made on the fluid obtained from cadavers and in consideration of this it must be remarked that this fluid quickly changes after death and that the results obtained therefore are not comparable with the fluid during life.

Aqueous Humor. This fluid is clear, alkaline toward litmus, and has a specific gravity of 1.003–1.009. The amount of solids is on an average 13 p. m., and the amount of proteins only 0.8–1.2 p. m. The protein consists of *seralbumin* and *globulin* and very little *fibrinogen* and *mucin*. According to GRUENHAGEN it contains *paralactic acid*, another dextrogyrate substance, and a *reducing body* which is unlike sugar or dextrin. PAUTZ [4] found *urea* and *sugar* in the aqueous humor of oxen.

Blister-fluid. The content of blisters caused by burns, and of vesicatory blisters and the blisters of the *pemphigus chronicus*, is generally a fluid rich in solids and proteins (40–65 p. m.). This is especially true of the contents of vesicatory blisters. In a burn-blister K. MÖRNER [5] found 50.31 p. m. proteins, among which were 13.59 p. m. globulin and 0.11 p. m. fibrin. The fluid contains a substance which reduces copper oxide, but no pyrocatechin. The fluid of the pemphigus is alkaline in reaction. A wound secretion collected by LIEBLEIN [6] under aseptic conditions was alkaline in reaction, and contained less protein than the blood-serum. It formed a slight fibrin clot, and contained proteoses only at first or at the beginning of the abscess formation. As the wound healed, the relation between the globulin and albumin changed, and on

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[1] See Salkowski, l. c. New quantitative analyses of cerebrospinal and hydrocephalus fluids may be found in the cited works of Nawratzki, Panzer, and Salkowski.

[2] Bioch. Zeitschr., **9**.

[3] See Maly's Jahresber., **22**, 346, and Centralbl. f. Physiol., **15**, 216.

[4] Gruenhagen, Pflüger's Arch., **43**; Pautz. Zeitschr. f. Biologie, **31**.

[5] Skand. Arch. f. Physiol., **5**.

[6] Habilitationsschrift Prag. 1902, printed by H. Laupp, Tübingen.

the third day of the healing the quantity of albumin was at least nine-tenths of the total protein.

The fluid of **subcutaneous œdema.** This is, as a rule, very poor in solids, purely serous, does not contain fibrinogen, and has a specific gravity of 1.005–1.013. The quantity of proteins is in most cases lower than 10 p. m.—according to HOFFMANN 1–8 p. m.—and in serious affections of the kidneys, generally with amyloid degeneration, less than 1 p. m. has been shown (HOFFMANN [1]). The œdematous fluid also habitually contains *urea*, 1–2 p. m., and *sugar*.

The FLUID OF THE ECHINOCOCCUS cyst is related to the transudates, and is poor in proteins. It is thin and colorless, and has a specific gravity of 1.005–1.015. The quantity of solids is 14–20 p. m. The chemical constituents are *sugar* (2.5 p. m.), *inosite*, traces of *urea*, *creatine*, *succinic acid*, and salts (8.3–9.7 p. m.). Proteins are found only in traces, and then only after an inflammatory irritation. In the last-mentioned case 7 p. m. proteins have been found in the fluid.

The Synovial Fluid and Fluid in Synovial Cavities around Joints, etc. The synovia is hardly a transudate, but it is often discussed in an appendix to the transudates.

The synovia is an alkaline, sticky, fibrous, yellowish fluid which is cloudy, from the presence of cell-nuclei and the remains of destroyed cells, but is also sometimes clear. Besides *proteins* and salts, it also contains a mucin substance, *synoviamucin* (v. HOLST [2]). In pathological synovia, HAMMARSTEN found a mucin-like substance which is not mucin. It behaves like a nucleoalbumin or a nucleoprotein, and gives no reducing substance on boiling with acids. SALKOWSKI [3] also found a mucin-like substance in a pathological synovial fluid, which was neither mucin nor nucleoalbumin. He called the substance *synovin*.

The composition of synovia is not constant, but is different in rest and in motion. In the last-mentioned case the quantity of fluid is less, but the amount of the mucin-like body, of proteins, and of the extractive bodies is greater, while the quantity of salts is diminished. This may be seen from the following analyses by FRERICHS.[4] The figures represent parts per 1000.

	I. Synovia from a Stall-fed Ox.	II. Synovia from a Field-fed Ox.
Water	969.9	948.5
Solids	30.1	51.5
Mucin-like body	2.4	5.6
Albumin and extractives	15.7	35.1
Fat	0.6	0.7
Salts	11.3	9.9

[1] Deutsch. Arch. f. klin. Med., **44**.

[2] Zeitschr. f. physiol. Chem., **43**.

[3] Hammarsten, Maly's Jahresber., **12**; Salkowski, Virchow's Arch., **131**.

[4] Wagner's Handwörterbuch, **3**, Abt. 1, 463.

The synovia of new-born babes corresponds to that of resting animals. The fluid of the bursæ mucosæ, as also the fluid in the synovial cavities around joints, etc., is similar to synovia from a qualitative standpoint.

III. PUS.

Pus is a yellowish-gray or yellowish-green, creamy mass of a faint odor and an unsavory, sweetish taste. It consists of a fluid, the *pus-serum*, in which solid particles, the *pus-cells*, swim. The number of these cells varies so considerably that the pus may at one time be thin and at another time so thick that it scarcely contains a drop of serum. The specific gravity, therefore, may also greatly vary, namely, between 1.020 and 1.040, but ordinarily it is 1.031–1.033. The reaction of fresh pus is generally alkaline, but it may become neutral or acid from a decomposition in which fatty acids, glycerophosphoric acid, and also lactic acid are formed.

In the chemical investigation of pus, the pus-serum and the pus-corpuscles must be studied separately.

Pus-serum. Pus does not coagulate spontaneously nor after the addition of defibrinated blood. The fluid in which the pus-corpuscles are suspended is not to be compared with the blood-plasma, but rather with the serum. The pus-serum is pale yellow, yellowish-green, or brownish-yellow, and has an alkaline reaction toward litmus. It contains, for the most part, the same constituents as the blood-serum; but sometimes besides these—when, for instance, the pus has remained in the body for a long time—it contains a nucleoalbumin or a nucleoprotein which is precipitated by acetic acid and is soluble with great difficulty in an excess of the acid (*pyin* of the earlier authors). This nucleoalbumin seems to be formed from the hyaline substance of the pus-cells by maceration. The pus-serum contains, moreover, at least in many cases, no fibrin ferment. According to the analyses of HOPPE-SEYLER [1] the pus-serum contains in 1000 parts:

	I.	II.
Water	913.70	905.65
Solids	86.30	94.35
Proteins	63.23	77.21
Lecithin	1.50	0.56
Fat	0.26	0.29
Cholesterin	0.53	0.87
Alcohol extractives	1.52	0.73
Water extractives	11.53	6.92
Inorganic salts	7.73	7.77

[1] Med.-Chem. Untersuch., 490.

The ash of pus-serum has the following composition, calculated to 1000 parts of the serum:

	I.	II.
NaCl	5.22	5.39
Na_2SO_4	0.40	0.31
Na_2HPO_4	0.98	0.46
Na_2CO_3	0.49	1.13
$Ca_3(PO_4)_2$	0.49	0.31
$Mg_3(PO_4)_2$	0.19	0.12
PO_4 (in excess)	....	0.05

The **pus-corpuscles** are generally thought to consist chiefly of emigrated white blood-corpuscles, and their chemical properties have therefore been given in discussing these. The molecular granules, fat-globules, and red blood-corpuscles are considered rather as casual form-elements.

The pus-cells may be separated from the serum by centrifugal force, or by decantation directly or after dilution with a solution of sodium sulphate in water (1 vol. saturated sodium-sulphate solution and 9 vols. water) and then washed by this same solution in the same manner as the blood-corpuscles.

The chief constituents of the pus-corpuscles are proteins, of which the largest portion seems to be a nucleoprotein which is insoluble in water and which expands into a tough, slimy mass when treated with a 10-per cent common-salt solution. This protein substance, which is soluble in alkali but is quickly changed thereby, is called ROVIDA's *hyaline substance*, and the property of the pus of being converted into a slime-like mass by a solution of common salt depends on this substance. Besides this substance, to which the nucleoprotein of the pus-cells investigated by STRADA [1] seems to stand in close relation, we also have a *globulin* which coagulates at 48–49° C., as well as *serglobulin* (?), *seralbumin*, a substance similar to coagulated protein (MIESCHER), and lastly *peptone* or *proteose* (HOFMEISTER [2]). It is very remarkable that no nucleo-histone or histone has been detected in the pus-cells, although histone occurs in the cells of the lymph glands.

There are also found in the protoplasm of the pus-cells, besides the proteins, *lecithin*, *cholesterin*, *glucothionic acid*,[3] *purine bodies*, *fat*, and *soaps*. HOPPE-SEYLER has found *cerebrin*, a decomposition product of a protagon-like substance, in pus (see Chapter XI). KOSSEL and FREYTAG [4] have isolated from pus two substances, *pyosin* and *pyogenin*, which

[1] Bioch. Zeitschr., **16**.

[2] Miescher in Hoppe-Seyler's Med.-Chem. Untersuch., 441; Ch. Pons. Maly's Jahresb., **39**; Hofmeister, Zeitschr. f. physiol. Chem., **4**.

[3] Mandel and Levene, Bioch. Zeitschr., **4**.

[4] Zeitschr. f. physiol. Chem., **17**, 452.

belong to the cerebrin group (see Chapter XI). HOPPE-SEYLER[1] claims that *glycogen* appears only in the living, contractile white blood-cells and not in the dead pus-corpuscles. Several other investigators have, nevertheless, found glycogen in pus. The cell-nucleus contains *nuclein* and *nucleoproteins*.

In regard to the occurrence of *enzymes* in the pus-cells it must be remarked that neither thrombin nor prothrombin is found therein, although these bodies are generally considered as being derived from the leucocytes, and also obtainable from the thymus leucocytes. The occurrence in the pus-cells, besides catalases and oxidases, of a proteolytic enzyme, is of great interest. It is not only important for the intracellular digestion and for the amount of proteoses in the pus-cell, but also for the solution of the fibrin clot and pneumonic infiltrations (FR. MÜLLER, O. SIMON[2]). A lipase, which splits neutral fats, also occurs, according to FIESSINGER and MARIE, in pus.

The *mineral constituents* of the pus-corpuscles are potassium, sodium, calcium, magnesium, and iron. A part of the alkalies exists as chlorides, and the remainder, as well as the chief part of the other bases, exists as phosphates.

The quantitative composition of the pus-cells from the analyses of HOPPE-SEYLER is as follows, in parts per 1000 of the dried substance:

	I.		II.
Proteins	137.62	685.85	673.69
Nuclein	342.57		
Insoluble bodies	205.66		
Lecithin	143.83		75.64
Fat			75.00
Cholesterin	74.00		72.83
Cerebrin	51.99		102.84
Extractive bodies	44.33		

MINERAL SUBSTANCES IN 1000 PARTS OF THE DRIED SUBSTANCE.

NaCl	4.34
$Ca_3(PO_4)_2$	2.05
$Mg_3(PO_4)_2$	1.13
$FePO_4$	1.06
PO_4	9.16
Na	0.68
K	Traces (?)

MIESCHER obtained other results for the alkali compounds, namely, potassium phosphate 12, sodium phosphate 6.1, earthy phosphate and iron phosphate 4.2, sodium chloride 1.4, and phosphoric acid combined with organic substances 3.14–2.03 p. m.

In pus from congested abscesses which has stagnated for some time there occur *peptone* (proteose), *leucine* and *tyrosine*, free *fatty acids* and

[1] Hoppe-Seyler, Physiol. Chem., 790.

[2] Fr. Müller, Verhandl. Nat. Gesellsch. zu. Basel, 1901; O. Simon, Deutsch. Arch. f. klin. Med., 70.

volatile fatty acids, such as formic acid, butyric acid and valeric acid. There are also found *urea*, *glucose* (in diabetes), *bile-pigments*, and *bile-acids* (in catarrhal icterus).

As more specific but not constant constituents of the pus must be mentioned the following: *pyin*, which seems to be a nucleoprotein precipitable by acetic acid, and also *pyinic acid* and *chlorrhodinic acid*, which have been so little studied that they cannot be more fully treated here.

In many cases a blue, more rarely a green, color, has been observed in the pus. This depends on the presence of micro-organisms (*Bacillus pyocyaneus*). From such pus FORDOS and LÜCKE [1] have isolated a crystalline blue pigment, *pyocyanin*, and a yellow pigment, *pyoxanthose*, which is produced from the first by oxidation.

Appendix.

Lymphatic Glands, Spleen and Endocrinic Glands.

The Lymphatic Glands. The cells of the lymphatic glands are found to contain the protein substances generally occurring in cells (Chapter V). According to BANG [2] they also contain histone nucleates (*nucleohistone*), but in smaller amounts and of a different variety from the better-studied nucleohistone from the thymus gland. Proteoses occur as products of autolysis. By a lengthy autolysis of lymph glands REH [3] found ammonia, tyrosine, leucine (somewhat scanty), thymine, and uracil among the cleavage products. Besides the other ordinary tissue constituents, such as collagen, reticulin, elastin, and nuclein, there occur in the lymphatic glands also *cholesterin*, *fat*, *glycogen*, *sarcolactic acid*, *purine bases*, and *leucine*. In the inguinal glands of an old woman OIDTMANN found 714.32 p. m. water, 284.5 p. m. organic and 1.16 p. m. inorganic substances. In the cells of the mesenteric lymphatic glands of oxen, BANG [4] found 804.1 p. m. water, 195.9 p. m. solids, 137.9 total proteins, 6.9 p. m. histone nucleate, 10.6 p. m. nucleoprotein, 47.6 p. m. bodies soluble in alcohol, and 10.5 p. m. mineral constituents.

The Thymus. The cells of this gland are very rich in nuclein bodies and relatively poor in the ordinary proteins, but their nature has not been closely studied. The chief interest is attached to the nuclein substances. KOSSEL and LILIENFELD first prepared from the watery extract of the gland, by precipitating with acetic acid and then further purifying, a

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[1] Fordos, Compt. Rend., 51 and 56; Lücke, Arch. f. klin. Chirurg., 3; Boland, Centralbl. f. Bakt. u. Parasit., I., 25.

[2] Studier over Nucleoproteider, Kristiania, 1902, and Hofmeister's Beiträge, 4.

[3] Hofmeister's Beiträge, 3.

[4] l. c.

protein substance which has been generally called *nucleohistone*. By the action of dilute hydrochloric acid upon nucleohistone it splits, according to these investigators, into *histone* and *leuconuclein*. The leuconuclein is a true nuclein; hence it is a nucleic-acid compound with protein which is relatively poor in protein and rich in phosphorus. The more recent investigations of BANG, MALENGREAU, HUISKAMP and GOUBAN[1] upon nucleohistone all show that this nucleoprotein is not a unit substance, but a mixture of at least two bodies. The views of the investigators mentioned differ quite essentially from one another as to the nature of these bodies, but this is partly due to the different methods used by them and partly to the ready changeability of the substances in question.

Besides the real nucleohistone, B-nucleoalbumin of MALENGREAU, LILIENFELD'S histone contains a second nucleoprotein which BANG and HUISKAMP call simple nucleoprotein, while MALENGREAU designates it A-nucleoalbumin. This protein, which contains only about 1 per cent phosphorus and which is possibly identical with the nucleoprotein found by LILIENFELD in the thymus, yields a nuclein, but no free nucleic acid, on cleavage. As a second cleavage product it yields, according to MALENGREAU, the A-histone, which can be readily precipitated by magnesium and ammonium sulphates from the ordinary B-histone of the thymus gland. The occurrence of A-histone in the gland has been verified by BANG, and according to BANG and HUISKAMP the A-histone is not derived from the nucleoprotein, as these investigators claim that it yields no histone. According to BANG the nucleoprotein yields only an albuminate, besides the nuclein, as cleavage products. According to GOUBAN we have been dealing with three substances, namely a nucleoprotein which does not yield any histone, and two nucleohistones, which correspond to the nucleoalbumins A and B of MALENGREAU and form the mixture of lime-nucleohistone of HUISKAMP. They occur in this last mentioned mixture in a somewhat modified form due to the method of preparation.

The true nucleohistone, which is much richer in phosphorus (the calcium salt containing, according to BANG, on an average 5.23 per cent P), yields ordinary histone (or 2 histones) as one cleavage product and free nucleic acid as the other. According to BANG, whose statements on this point have been substantiated by MALENGREAU, it splits on saturating with NaCl into nucleic acid and histone without yielding any other protein. On this account BANG does not consider this body as nucleohistone in the ordinary sense, i.e., not as a nucleoprotein, but as a histone

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[1] Lilienfeld, Zeitschr. f. physiol. Chem., **18**; Kossel, *ibid.*, **30** and **31**; Bang., *ibid.* **30** and **31**. See also Arch. f. Math. og Naturvidenskab, **25**, Kristiania, 1902, and Hofmeister's Beiträge, 1 and 4; Malengreau, La Cellule, **17** and **19**; Huiskamp, Zeitschr. f. physiol. Chem., **32**, **34** and **39**; Gouban, Bioch. Centralbl., **9**, 803.

nucleate. We cannot say whether this applies to the two nucleohistones (if there are two). The nucleohistone or mixture of nucleohistones behave like an acid, whose salts, especially the calcium salt, have been closely studied by HUISKAMP. On the electrolysis of a solution of alkali nucleohistone in water HUISKAMP also found that the nucleohistone collected in traces at the anode, and that the sodium compound is therefore ionized in the solution. The nucleic acid-calcium histone-compound has been prepared, it seems, in a pure state by BANG, and he found the following average composition: C 43.69; H 5.60; N 16.87; S 0.47; P 5.23; Ca 1.71 per cent.

The nucleohistone prepared by HUISKAMP'S method of precipitating with $CaCl_2$ is, according to him, a mixture of two nucleohistones, of which one, the α-nucleohistone, contains 4.5 per cent phosphorus, and the other, β-nucleohistone contains, on the contrary, only in round numbers 3 per cent phosphorus.[1] As the two nucleohistones are poorer in phosphorus than the nucleic acid-histone compound analyzed by BANG, and as HUISKAMP on cleavage of his preparation did not, like BANG and MALENGREAU, obtain pure nucleic acid, it is still a question whether HUISKAMP was working with sufficiently pure substances.

In regard to the methods used by the above investigators in the isolation of the bodies in question we must refer to the original publications.

In connection with the so-called nucleohistone, attention must be called to *tissue fibrinogen* and *cell fibrinogen*, which are compound proteins, and are claimed by certain investigators to stand in close relation to the coagulation of the blood. These may be in part nucleoproteins and in part also nucleohistones. To this same group belong also the important cell constituents described by ALEX. SCHMIDT[2] and called *cytoglobin* and *preglobulin*. The cytoglobin, which is soluble in water, may be considered as the alkali compound of preglobulin. The residue of the cells left after complete extraction with alcohol, water, and salt solution has been called *cytin* by ALEX. SCHMIDT.

Besides the above-mentioned and the ordinary bodies belonging to the connective-tissue group, small quantities of *fat*, *leucine*, *succinic acid*, *lactic acid*, *sugar*, and traces of *iodothyrin* are present. According to GAUTIER[3] *arsenic* also occurs in very small amounts, and no doubt here as well as in other organs it is related to the nuclein substances. The richness in nuclein bodies explains the occurrence of large quantities of *purine bases*, chiefly *adenine*, whose quantity, according to KOSSEL and SCHINDLER,[4] is 1.79 p. m. in the fresh organ and 19.19 p. m. in the dry substance, and *guanine*. The bodies *thymine* and (*uracil?*) obtained, besides lysine and ammonia, by KUTSCHER, as products of autodigestion of the gland, probably have a similar origin. Among the enzymes,

[1] Zeitschr. f. physiol. Chem., **39**.
[2] See footnote 1, p. 307.
[3] Compt. Rend., **129**.
[4] Zeitschr. f. physiol. Chem., **18**; Kutscher, Zeitschr. f. physiol. Chem., **24**.

besides *arginase*, *guanase*, *adenase*, and *proteolytic* enzyme we must especially mention the enzyme studied by JONES,[1] which acts like a *nuclease*, splitting off phosphoric acid and purine bases, from the nucleoproteins. This enzyme, contrary to trypsin, acts best in acid liquids, and is readily destroyed by alkalies at body temperature. The quantitative composition of the lymphocytes from the thymus of a calf is, according to LILIENFELD'S analysis, as follows. The results are given in 1000 parts of the dried substance:

Proteids	17.7
Leuconuclein	687.9
Histone	86.7
Lecithin	75.1
Fat	40.2
Cholesterin	44.0
Glycogen	8.0

The dried substance of the leucocytes amounted to an average of 114.9 p. m. Potassium and phosphoric acid are prominent mineral constituents. LILIENFELD found KH_2PO_4 among the bodies soluble in alcohol.

Attention must be called to the analyses of BANG,[2] which show that the thymus contains about the same quantity of nucleoprotein, but about five times as much histone nucleate as the lymphatic glands—calculated in both cases upon the same amount of dry substance. OIDTMAN[3] found 807.06 p. m. water, 192.74 p. m. organic and 0.2 p. m. inorganic substances in the gland of a child two weeks old.

In regard to the functions of the thymus it seems to be the general view that this gland takes part in the recruiting of the blood lymphocytes and correspondingly belong to the lymphoid organs. On the other hand also certain other observations indicate that it may belong to the endocrinic organs. It is generally admitted that the extirpation of the thymus leads to a reduction and change in the formation of bone. A certain relation also exists with the organs of generation and perhaps a reciprocal action also exists between it and other organs with internal secretion.

The Spleen. The pulp of the spleen cannot be freed from blood. The mass which is separated from the spleen capsule and the structural tissue by pressure, and which ordinarily serves as material for chemical investigations is, therefore a mixture of blood and spleen constituents. For this reason the proteins of the spleen are little known. The nucleoprotein isolated by LEVENE and MANDEL[4] is to be considered as a true

[1] Zeitschr. f. physiol. Chem., 41.

[2] l. c., Arch. f. Math., etc.

[3] Cited from v. Gorup-Besanez, Lehrb. d. physiol. Chem., 4. Aufl., p. 732.

[4] Bioch. Zeitschr., 5.

spleen constituent, and this nucleoprotein yields 25 per cent glutamic acid on hydrolysis. Histone has not been directly detected in the spleen; but its presence is to be admitted because KRASNOSSELSKY [1] was able to isolate a histone-peptone as sulphate from the spleen. The *ferruginous albuminate* has been considered as a spleen constituent for a long time, and especially also a protein substance which does not coagulate on boiling and which is precipitated by acetic acid and yields an ash containing much phosphoric acid and iron oxide. This substance is probably identical with the nucleoproteins which later investigators such as SATO and CAPEZZUOLI [2] have prepared from the spleen. These nucleoproteins, which are modified products, contain iron in variable amounts and more or less firmly combined.

The pulp of the spleen, when fresh, has an alkaline reaction, but quickly turns acid, due partly to the formation of free *paralactic acid* and partly perhaps to *glycerophosphoric acid*. Besides these two acids there are found in the spleen also *volatile fatty acids*, as formic, acetic, and butyric acids, as well as *succinic acid*, *neutral fats*, *cholesterin*, traces of *leucine*, *inosite* (in ox-spleen), *scyllite*, a body related to inosite (in the spleen of Plagiostoma), *glycogen* (in dog-spleen), *uric acid*, *purine bases*, and *jecorin*. LEVENE found a *glucothionic acid* in the spleen, i.e., an acid which is related to chondroitin-sulphuric acid but not identical therewith, and which gives a beautiful violet coloration with orcin and hydrochloric acid. The question whether this glucothionic acid originates from the above-mentioned nucleoprotein or from the mucoid substance has not been decided (LEVENE and MANDEL). In regard to the question whether this acid is a unit body or not we refer to the work of MANDEL and NEUBERG and LEVENE and JACOBS.[3]

In the human and ox-spleen BUROW [4] has found three phosphatides which all contain iron in organic combination. Among these one is a saturated diaminomonophosphatide and the other two are unsaturated phosphatides.

Many enzymes are found in the spleen also, and certain of these are of special interest. To these belong the uric-acid-forming enzyme, the *xanthine oxidase* (BURIAN), which occurs in the spleen of many animals, but not in man, and which transforms the oxypurines, hypoxanthine, and xanthine into uric acid; also the deamidizing enzymes

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[1] Zeitschr. f. physiol. Chem., **49**.

[2] Sato, Bioch. Zeitschr., 22; Capezzuoli; Zeitschr. f. physiol. Chem., **60**.

[3] Levene, Zeitschr. f. physiol. Chem., **37**; Levene and Mandel, *ibid.*, **45** and **47**; Mandel and Neuberg, Bioch. Zeitschr., **18**; Levene, *ibid.*, **16**; Neuberg, *ibid.*, **16**; Levene and Jacobs, Journ. of experim. Medic., **10**.

[4] Bioch. Zeitschr., **25**.

guanase and *adenase* (LEVENE, SCHITTENHELM, JONES and PARTRIDGE, JONES and WINTERNITZ), by the first of which the guanine is transformed into xanthine, and by the latter the adenine into hypoxanthine. The guanase also occurs in the spleen of the ox and horse, but not (JONES), or only in small amounts (SCHITTENHELM), in the pig-spleen.[1] The spleen also contains two enzymes, *lienases*, as shown by HEDIN (and ROWLAND), one of which, the α-lienase, acts chiefly in alkaline solution, while the other, β-lienase, is active only in acid reaction. These enzymes, which without doubt stand in close relation to the leucocytes, not only act autolytically upon the proteins of the spleen, but they also dissolve fibrin and coagulated blood-serum. The spleen also contains *nucleases* and besides, as TANAKA[2] has found for the pig-spleen, diastase, invertin, lipase, urease, trypsin and an erepsin like enzyme.

Among the constituents of the spleen the *deposit rich in iron*, which consists of ferruginous granules or conglomerate masses of them, and which is derived from a transformation of the red blood-corpuscles, is of special interest. It was closely studied by NASSE. This deposit does not occur to the same extent in the spleen of all animals. It is found especially abundant in the spleen of the horse. NASSE[3] on analyzing the grains (from the spleen of a horse) obtained 840–630 p. m. organic and 160–370 p. m. inorganic substances. These last consisted of 566–726 p. m. Fe_2O_3, 205–388 p. m. P_2O_5, and 57 p. m. earths. The organic substances consisted chiefly of proteins (660–800 p. m.), nuclein (52 p. m. maximum), a yellow coloring-matter, extractive bodies, fat, cholesterin, and lecithin.

In regard to the *mineral constituents*, it is to be observed that the amount of iron in new-born and young animals is small (LAPICQUE, KRÜGER, and PERNOU), in adults more appreciable, and in old animals sometimes very considerable. NASSE found nearly 50 p. m. iron in the dried pulp of the spleen of an old horse. GUILLEMONAT and LAPICQUE[4] have determined the iron in man. They find no regular increase with growth, but in most cases 0.17–0.39 p. m. (after subtracting the blood-iron) calculated on the fresh substance. A remarkably high amount of iron is not dependent upon old age, but is a residue from chronic diseases. MAGNUS-LEVY found 0.72 p. m. iron in the fresh human spleen.

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[1] See Chapter XIV for the literature.

[2] Hedin and Rowland, Zeitschr. f. physiol. Chem., **32**, and Hedin, Journ. of Physiol., **30**, and Hammarsten's Festschr., 1906; Tanaka, Bioch. Zeitschr, **37**.

[3] Maly's Jahresber., **19**, p. 315.

[4] Lapicque, *ibid.*, **20**; Lapicque and Guillemonat, Compt, rend. de soc. biol., 48, and Arch de Physiol. (5) 8; Krüger and Pernou, Zeitschr. f. Biologie, **27**; Nasse, cited from Hoppe-Seyler, Physiol. Chem., 720.

On the analysis of the human spleen MAGNUS-LEVY found 784.7 parts water, 215.3 parts solids, 27.7 parts fat and 27.9 parts nitrogen in 1000 parts of the fresh organ. In the dog spleen, CORPER [1] found 750 to 770 p. m. water, and 120–150 p. m. ether soluble substances, of which one-fourth consisted of cholesterin and three-fourths of lecithin. As purine bases he found 1.1 p. m. guanine, 0.6 p. m. adenine, 0.15 p. m. hypoxanthine and 0.04 p. m. xanthine.

In regard to the pathological processes going on in the spleen we must specially recall the abundant re-formation of leucocytes in leucæmia and the appearance of amyloid substance (see page 172).

The physiological functions of the spleen are little known, with the exception of its importance in the formation of leucocytes. Some consider the spleen as an organ for the dissolution of the red blood-corpuscles, and the occurrence of the above-mentioned deposit rich in iron seems to confirm this view, but this iron could in part have another origin. ASHER and his collaborators GROSSENBACHER, ZIMMERMANN and H. VOGEL have found that the spleen is an organ for the iron metabolism, as they found in a splenectomized dog that the iron elimination was much greater than in a dog with its spleen. R. BAYER [2] has made a similar observation on a splenectomized human being, and the spleen it seems has the purpose of retaining for the organism the iron set free in the metabolism and also in starvation metabolism.

The spleen has also been claimed to play a certain part in digestion especially in pancreatic digestion. This organ is said by SCHIFF, HERZEN, and others to be of importance in the production of trypsin in the pancreas. The investigations of HERZEN seem to confirm this relation, but the recent work of PRYM [3] has made the assumption doubtful.

Splenectomized dogs require according to RICHET [4] for their maintenance more food, about one-third more, than normal dogs. The spleen makes a complete utilization of the food possible or diminishes its consumption.

An increase in the quantity of uric acid eliminated in splenic leucæmia has been observed by many investigators (see Chapter XIV), while the reverse has been observed under the influence of quinine in large doses, which produces an enlargement of the spleen. These facts give a rather positive proof that there is a close relation between the spleen and the

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[1] Magnus-Levy, Bioch. Zeitschr., **24**; H. J. Corper, Journ. of biol. Chem., **11**.

[2] Asher and Grossenbacher, Centralbl. f. Physiol.. **22**, 375, and Bioch. Zeitschr, **17**; Zimmermann, Bioch. Zeitschr., **17**; R. Bayer, Bioch. Centralbl., **9**, 815.

[3] Schiff, cited by Herzen, Pflüger's Arch., **30**, 295, 308, and **84**, and Maly's Jahresber., 18; Prym, Pflüger's Arch., **104** and **107**; see also Chapter VIII.

[4] Journ. de Physiol. et de Pathol. gén., **14** and **15**.

formation of uric acid. This relation has been studied by HORBACZEWSKI. He has shown that when the spleen-pulp and blood of calves are allowed to act on each other, under certain conditions and certain temperature, in the presence of air, large quantities of uric acid are formed, and he has also shown that the uric acid originates from the nucleins of the spleen.[1] This behavior is explained by the above-mentioned investigations of BURIAN, SCHITTENHELM, JONES, and others on the enzymotic formation of uric acid, and the deamidization of the purine bodies, and a relation between the spleen and uric-acid formation is indisputable. Still we cannot say that the spleen shows a special relation to the uric-acid formation as compared with other organs (see Chapter XIV).

The spleen has the same property as the liver of retaining foreign bodies, metals and metalloids.

The Thyroid Gland. The nature of the different protein substances occurring in the thyroid gland has not been sufficiently studied, but at present, through the researches of OSWALD, there are known at least two bodies which are constituents of the so-called secretion of the glands, the colloids. One of these, *iodothyreoglobulin*, behaves like a globulin, while the other is a nucleoprotein (see also GOURLAY[2]). The iodine present in the gland occurs chiefly in the first body, while the arsenic, which has been shown to be a normal constituent by GAUTIER and BERTRAND,[3] seems to be related to the nuclein substances.

According to OSWALD the iodothyreoglobulin occurs only in those glands which contain colloid, while the colloid-free glands, the parenchymatous goitre, and the glands of the new-born contain thyreoglobulin free from iodine. The thyreoglobulin first becomes iodized into iodothyreoglobulin on passing from the follicle-cells. Besides these mentioned bodies *leucine*, *xanthine*, *hypoxanthine*, *choline*, *iodothyrine*, *lactic* and *succinic acids* occur in the thyreoidea. Like certain other organs, substances also occur in the thyroid which act upon the blood pressure and indeed partly as vasodilator and partly depressing but whose chemical nature has not been positively established. Among the enzymes we find lipases and catalases which, according to JUSCHTSCHENKO,[4] are related to the corresponding enzymes of the blood. MAGNUS-LEVY[5] found 757 parts water, 243 parts solids, 43.8 parts fat, 26.8 parts nitrogen, and 0.058 parts iron in 1000 parts of the human thyroid gland.

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[1] Monatshefte f. Chem., **10**, and Wein. Sitzungsber. Math. Nat. Klasse, **100**, Abt. 3.

[2] Gourlay, Journ. of Physiol., **16**; Oswald, Zeitschr. f. physiol. Chem., **32**, and Biochem. Centralbl., **1**, 249.

[3] Gautier, Compt. Rend., **129**. See also *ibid.*, **130**, **131**, **134**, **135**; Bertrand, *ibid.*, **134**, **135**.

[4] Juschtschenko, Bioch. Zeitschr., 25 and Arch. scienc. biol. de St. Petersbourg, **15**.

[5] Bioch. Zeitschr., **24**.

In "STRUMACYSTICA" HOPPE-SEYLER found hardly any protein in the smaller glandular vessels, but an excess of *mucin*, while in the larger he found a great deal of *protein*, 70–80 p. m.[1] *Cholesterin* is regularly found in such cysts, sometimes in such large quantities that the entire contents form a thick mass of cholesterin plates. Crystals of *calcium oxalate* also occur frequently. The contents of the struma cysts are sometimes of a brown color, due to decomposed coloring-matter, *methæmoglobin* (and hæmatin?). Bile-coloring matters have also been found in such cysts. (In regard to the *paralbumins* and *colloids* which have been found in struma cysts and colloid degeneration, see Chapter XII.)

Those substances which bear a close relation to the functions of the gland seem to be of special interest.

The complete extirpation, as also the pathological destruction, of the thyroid gland causes great disturbances, ending finally in death. In dogs, after the total extirpation, a disturbance of the nervous and muscular systems occurs, such as trembling and convulsions, and death generally supervenes shortly after, most often during such an attack. The researches of GLEY, VASSALE and GENERALI[2] upon various animals have shown that for the success of the operation it is of the greatest importance whether the parathyroids, discovered by SANDSTRÖM,[3] are removed at the same time or not. In herbivora (rabbits) because of the anatomical relations, the parathyroids are seldom extirpated in the operation of the removal of the thyroid, the tetany does not regularly occur and the disturbance in metabolism is most striking. If these glands are not extirpated in dogs, the tetany also does not appear, and the disturbances in metabolism occur. In human beings, after the removal of the gland by operation, different disturbances appear, such as nervous symptoms, diminished intelligence, dryness of the skin, falling out of the hair, and, on the whole, those symptoms which are included under the name cachexia thyreopriva, death coming gradually. Among these symptoms must be mentioned the peculiar slimy infiltration and exuberance of the connective tissue called *myxœdema*.

All these conditions indicate that the thyroids belong to those glands with internal secretion, so called *endocrinic* glands. The most convincing proof of this is the fact that the ordinary symptoms do not occur if a small piece of the gland is allowed to remain in the body, or even when a piece of the gland is transplanted in any part of the body. The observations of ASHER and FLACK[4] that the irritation of the nerves of the thyroid causes an internal secretion from the thyroid gland into the blood, is of great interest in this connection. A further proof of practical

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[1] Physiol. Chem., p. 721.

[2] Gley, Compt. rend. soc. biol., 1891, and Arch. de Physiol (5), 4; Vassale and Generali, Arch. Ital. d. Biol., 25 and 26.

[3] Upsala Läkaref. Förh., 15 (1880).

[4] Asher and Flack, Zeitschr. f. Biol., 55.

importance is that the injurious results from removal of the thyroids can be counteracted by the introduction of artificial extracts of the thyroid gland into the body or by feeding with thyroid glands.

Of the disturbances in metabolism which occur on the extirpation or reduction of the thyroid function (athyreoidismus or hypothyreoidismus) we must especially mention the reduction in the protein catabolism which in a starving dog without thyroids may fall to about one-half of the starvation protein metabolism in a normal dog of the same size (FALTA and collaborators [1]). The reverse is observed when large quantities of the thyroid gland substance is fed, namely, a strong increase in the protein metabolism, besides certain other symptoms. BASEDOW'S disease is also considered as a form of hyperthyreoidismus which, by an increased activity of the glands, brings about an overproduction of the specific secretion. There does not seem to be any doubt that the thyroid glands stand in close relation to other endocrinic glands although for the present we are unable to survey this very complicated condition. One side of this reciprocal action with other organs, which is of special importance, is the relation of the thyroids to glycosuria, which will be discussed in a following chapter.

The glands with internal secretion, the so-called endocrinic glands, to which the adrenals belong, which will be discussed below, and the hypophysis, are of especially great interest because of the reciprocal action which they exert among each other and with other organs. A chemical correlation exists between different organs, of a kind, that bodies which are formed in one organ can awaken or regulate the functions of another organ or other organs. These chemically active substances, which awaken or regulate the activity of other organs have been given the group name *hormone* ($\delta\rho\mu\alpha\omega$ = I awaken or excite) by STARLING and to this group belong the specifically active constituents of the endocrinic glands.

It is impossible for the present to state anything about the kind of bodies having a specific action in the thyroid gland or anything about the importance of the bases found by certain investigators, such as S. FRÄNKEL, DRECHSEL, and KOCHER,[2] as these bodies have not been characterized sufficiently. It seems proved that the specifically active substance is, as first shown by NOTKIN[3] and OSWALD,[4] a protein substance: NOTKIN'S *thyreoproteid*, OSWALD'S *thyreoglobulin*. This does not conflict with the views

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[1] Eppinger, Falta and Rudinger, Zeitschr. f. klin. Med., **66**.

[2] Fränkel, Wein. med. Blätter, 1895 and 1896; Drechsel and Kocher, Centralbl. f. Physiol., **9**, 705.

[3] Wien. med. Wochenschr., 1895, and Virchow's Arch., **144**, Suppl., 224.

[4] Zeitschr. f. physiol. Chem., **32**, and Bioch. Centralbl., **1**, 249.

of BAUMANN and Roos that the active substance is *iodothyrin*, as this can be produced as a cleavage product from the iodothyreoglobulin. In fact OSWALD has found in the tryptic digestion of iodothyreoglobulin that a substance similar to iodothyrin is produced; important investigations [1] nevertheless make it probable that the thyreoglobulin is the active substance and not the iodothyrin. There are several reasons why the action of the thyroid gland substance is not due to one substance, but to several.

Iodothyrin is considered by BAUMANN, who first showed that the thyroid contained iodine and who with Roos [2] proved the importance of this substance for the physiological activity of the gland, as the only active substance. By boiling the finely divided gland with dilute sulphuric acid BAUMANN obtained iodothyrin as an amorphous, brown mass, nearly insoluble in water but readily soluble in alkali and precipitated again by the addition of acid. The iodothyrin, which is not a unit body, has a variable content of iodine and is not a protein substance. According to v. FÜRTH and SCHWARZ it is probably a melanoid-like transformation product of the iodized protein of the gland produced by the action of the acid.

Thyreoglobulin or **iodothyreoglobulin** was obtained by OSWALD from the watery extract of the gland by half saturating with ammonium sulphate. It has the properties of the globulins and with the exception of the iodine content it has about the same composition as the proteins. The amount of iodine varies: 0.46 per cent in pigs, 0.86 per cent in oxen, and 0.34 per cent in man. In the iodothyreoglobulin of the ox, NÜRENBERG [3] found 0.59–0.86 per cent iodine and 1.83–2.0 per cent sulphur. In young animals, whose glands contain no iodine, the thyreoglobulin is iodine-free. Thyreoglobulin on taking up iodine is converted into iodothyreoglobulin. By introducing iodine salts the iodine content of the iodothyreoglobulin can be raised in living animals and thus the physiological activity increased (OSWALD). The amount of iodine in the gland is markedly dependent upon the food.

JOLIN has examined a large number of thyroid glands from healthy and diseased persons (in SWEDEN), for their iodine content. In 28 children, ages

[1] See Oswald. Arch. f. exp. Path. u. Pharm., **60**; Pick and Pineles, Zeitschr. f. exp. Path. u. Therap., **7**.

[2] In regard to this subject, see Baumann and Roos, Zeitschr. f. physiol. Chem., **21** and **22**; also Baumann, Münch. med. Wochenschr., 1896; Baumann and Goldmann, *ibid.*; Roos., *ibid.*; v. Fürth and Schwarz, Pflüger's Arch., **124**. An extensive review of the literature on the action of iodothyrin and the thyroid preparations can be found in Roos, Zeitschr. f. physiol. Chem., **22**, 18. In regard to their action in protein catabolism and in metabolism, see F. Voit, Zeitschr. f. Biologie, **35**; Schöndorff, Plüger's Arch., **67**, and Andersson and Bergman, Skand. Arch. f. Physiol., 8; Magnus-Levy, Zeitschr. f. klin. Med., **32**. In regard to the function of the thyroid gland see also Sw. Vincent, Innere Sekretion etc. Ergebnisse d. Physiol., **11**, 218–302.

[3] Bioch. Zeitschr., **16**.

varying between 1 and 10 years, he found an average of 0.28 p. m. iodine in the glands. In 108 normal glands above 10 years old or adults the iodine content varied with an average of 1.56 p. m. iodine. In glands from persons after using iodine preparations (34 cases) the iodine content was 2.56 p. m. The amount of silicic acid in normal thyroid glands was found by H. SCHULZ [1] to be on an average 0.084 p. m., calculated on the dry substance. In goitres from GREIFSWALD and ZÜRICH he found 0.175 and 0.434 p. m., respectively. There does not seem to be any connection between the silicic acid content of the drinking water and the occurrence of goitre.

We cannot enter into a discussion as to the various hypotheses and theories in regard to the mode of action of the constituents of the thyroids. In the tetany appearing after parathyroidectomy many investigators find an increased elimination of calcium, nitrogen and ammonia and the hypothesis has been suggested that the tetany depends upon an increased irritability of the nervous system due to lack of calcium. The fact as found by several experimenters that a diminished calcium content of the organs in question does not occur, speaks against this theory. On the contrary, it seems to be generally admitted that lime salts reduce or prevent the tetany and, according to FROUIN,[2] this depends upon the lime combining with the carbonic acid produced, which is the cause of the tetany. The tetany is produced at least from a poison which is formed only on the removal of the parathyroids or if it is regularly produced it is made harmless by these organs.

G. MANSFIELD and FR. MÜLLER [3] have made investigations in regard to the action of the thyroids upon protein metabolism which indicate that lack of oxygen acts as an excitant upon the thyroids and that the increased protein catabolism, which occurs to a mean degree with lack of oxygen, depends upon a hyperfunction of the thyroid glands brought on by this condition. With greater lack of oxygen besides this a general damage to the protoplasm of the body cells may occur.[4]

The Adrenal Bodies. Besides proteins, substances of the connective tissue, and salts, there occur in the suprarenal capsule *inosite*, purine bases, especially *xanthine* (OKER-BLOM), *phosphatides* and *glycerophosphoric acid*, which is probably a decomposition product of the latter. The earlier accounts of the occurrence of benzoic acid, hippuric acid, and bile-acids are, on the contrary, doubtful, and are not substantiated by recent investigations (STADELMANN [5]). The medullary substance

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[1] Jolin, Hammarsten's Festschr., 1906; H. Schulz, Bioch. Zeitschr., 46.

[2] Compt. Rend., 148.

[3] Pflüger's Arch., 143.

[4] A very complete discussion of the physiology of the thyroid gland and the pertinent literature may be found in Sw. Vincent, Ergebnisse der Physiologie, 11, 218–302.

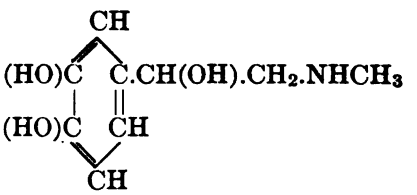
[5] Oker-Blom, Zeitschr. f. physiol. Chem., 28; Stadelmann, *ibid.*, 18, which also contains the literature on this subject.

contains the so-called chromaffine tissue, i.e., cells, whose substance is colored brown by chromic acid or chromates.

Earlier investigators, like VULPIAN and ARNOLD, have found, in the medulla, a *chromogen* which has been considered as connected with the abnormal pigmentation of the skin in ADDISON's disease. This chromogen, which is transformed by air, light, alkalies, iodine, and other bodies into a red pigment, seems, on the contrary, to be related to the substance *adrenalin*, of the gland which produces an increase in the blood-pressure. Choline has been shown to have a reverse effect upon this blood-pressure raising action, and LOHMANN has shown that it is formed in the cortical substance of the adrenals. In the cortical this last-mentioned experimenter[1] has found besides neurin, another not known base. That the watery extract of the adrenals has a blood-pressure raising action was shown by OLIVER and SCHÄFER, CYBULSKI and SZYMONOWICZ.[2] The substance which is here active was formerly called sphygmogenin and has also other actions besides bringing about a marked increase in blood-pressure by the strong contraction of the muscles of the periphery vessels; for instance, it can bring about glycosuria and mydriasis, especially in the frog's eye, has been chemically investigated by numerous experimenters.[3] v. FÜRTH calls it *suprarenin*, ABEL *epinephrin*. and TAKAMINE *adrenalin*. This last name seems to be the most generally accepted one.

Adrenalin (suprarenin epinephrin) (methylaminoethanolpyrocatechin)

$C_9H_{13}NO_3$, =



The constitution of adrenalin has been essentially proved by FRIEDMANN,[4] and he has shown the correctness of the above formula, which was given by PAULY. The synthesis of adrenalin, which was first performed by STOLZ,[5] is also in accordance with this formula. By the action of methylamine upon chloracetopyrocatechin we obtain methylaminoaceto-pyrocatechin:

$$C_6H_3(OH)_2.COCH_2Cl + NH_2CH_3 = C_6H_3(OH)_2.COCH_2.NHCH_3.HCl,$$

which yields adrenalin on reduction.

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[1] Centralbl. f. Physiol., 21, and Pflüger's Arch., 118 and Zeitschr. f. Biol., 56.

[2] Oliver and Schäfer, Proceed. of Physiol. Soc., London, 1895. Further literature on the function of the adrenals may be found in Sw. Vincent, Innere Sekretion, etc. Ergebnisse d. Physiol., 9, 505–585.

[3] The literature on this subject may be found in Abderhalden's Bioch. Handlexikon Bd. 5, s. 454–495.

[4] Hofmeister's Beiträge, 8.

[5] Ber. d. d. chem. Gesellsch., 37.

The synthetically prepared adrenalin is optically inactive *d-l*-adrenalin, while that from the adrenals is optically active *l*-adrenalin. FLÄCHER has divided the racemic adrenalin into the two optically active components, and the identity of the so-obtained synthetical adrenalin with the natural has been shown by ABDERHALDEN and FR. MÜLLER.[1] These last investigators also found that the *l*-adrenalin had at least 15 times as strong an action upon the blood-pressure as the *d*-adrenalin, and later ABDERHALDEN with THIES and SLAVU found that the *l*-adrenalin had also in other respects a much stronger action than *d*-adrenalin.

Adrenalin crystallizes in masses of needles or rhombic leaves. It is soluble in water, and can be precipitated from its solution by ammonia as a crystalline substance. Its aqueous solution containing hydrochloric acid is levorotatory: $(\alpha)_D = -50.72°$ (ABDERHALDEN and GUGGENHEIM [2]). On heating adrenalin it turns yellowish-brown at about 205° and decomposes at about 218° C. Its solution turns emerald green with ferric chloride in acid solution and carmine red in alkaline solution. Adrenalin reduces FEHLING'S solution and ammoniacal silver solution.

Among the reactions for adrenalin in solution we must especially mention the red coloration which is obtained on the addition of an oxidizing medium such as iodine or bi-iodate and dilute phosphoric acid and warming (FRÄNKEL and ALLERS), or of mercuric chloride in the presence of a catalyst such as the lime salts in tap-water (COMESATTI). These reactions are extremely delicate, 1:1000000–2000000. A still more delicate reaction (1:5000000) is the one suggested by EWINS,[3] namely a characteristic red coloration is obtained on adding a 0.1 per cent solution of potassium persulphate and warming gently in a boiling water-bath.

As above stated, it has been considered for some time that the color of the skin in ADDISON'S disease was connected with the adrenals or their chromogen. We know nothing positive in regard to this relation, but it is nevertheless of interest that pigments, and finally melanins or at least dark-brown substances, can be produced from adrenalin by the action of enzymes. NEUBERG has brought about such melanin formation by the extract from the metastases of a melanoma of the adrenals and also with the extract of the ink-sac of the sepia, and ABDERHALDEN and GUGGENHEIM [4] with tyrosinase. This would indicate a close relation

[1] Flächer, Zeitschr. f. physiol. Chem., **58**; Abderhalden and Franz Müller, *ibid.* **58**; with Thies, *ibid.*, **59**; with Slavu, *ibid.*, **59**; with Kautsch and Müller, *ibid.*, **61** and **62**; see also Fröhlich, Centralbl. f. physiol., **23** and Waterman, Zeitschr. f. physiol. Chem., **63**.

[2] Zeitschr. f. physiol. Chem., **57**.

[3] Fränkel and Allers, Bioch. Zeitschr., 18; Comesatti, Münch. med. Wochenschr. 1908 and Physiol. Centralbl., **23**; Ewins, Journ. of Physiol., **40**.

[4] Neuberg, Bioch. Zeitschr., 8; Abderhalden and Guggenheim, Zeitschr. f. physiol. Chem., **57**.

between adrenalin and tyrosine, which also gives melanin with the sepia enzyme, and indeed tyrosine has been considered as the probable mother-substance of adrenalin (HALLE). The investigations of EWIN'S and LAIDAW [1] to prove this last-mentioned possibility have not given any support thereto.

Besides the action of producing a rise in the blood-pressure, adrenalin is also of special interest because, as first shown by BLUM,[2] it also has a glycosuric action. We will discuss the question of adrenalin glycosuria and the relation which seems to exist between the internal secretions of the thyroids, the adrenals and the pancreas, when we treat of the formation of sugar and pancreas diabetes. We cannot here enter into the question of the reciprocal action between the adrenals and the other organs.

The hypophysis or pituitary gland has been little studied from a chemical standpoint. An extract of the gland shows, by its action, a certain similarity to an extract of the adrenals in that it causes a rise in blood pressure and by causing a dilation of the pupils of the frog's eye. Still no adrenalin could be detected in the gland. Also no iodine occurs in the glands (WELLS, DENIS [3]).

The gland consists essentially of two parts, one an outside formation of vascular-glandular epithelium and a lower nervous part the infundibular part. The outside part seems to have a relation to the growth of the tissues and skeleton and acromegalie and gigantism are claimed by many investigators to be related to this part. The infundibular part, on the contrary, contains the specific bodies which raises the blood-pressure and stimulates the smooth muscles of the uterus and upon the kidney secretion. The relation of the hypophysis to other endocrinic glands is still very much disputed.

[1] Halle, Hofmeister's Beiträge 8; Ewins and Laidaw, Journ. of Physiol., **40.**

[2] Deutsch. Arch. f. klin. Med., **91** and Pflüger's Arch., **90.**

[3] H. G. Wells, Journ. of biol. Chem., **7**; W. Denis, *ibid.*, **9.**

CHAPTER VII.

THE LIVER.

THE liver, which is the largest gland of the body, stands in close relation to the glands mentioned in Chapter VI. The importance of this organ for the assimilation of the food-stuffs and for the physiological composition of the blood is evident from the fact that the blood coming from the digestive tract, laden with absorbed bodies, must circulate through the liver before it is driven by the heart through the different organs and tissues. An assimilation of food-stuffs in the liver has been positively shown in the first place for carbohydrates in that the liver constructs a polysaccharide glycogen from hexoses, which according to the needs is then again retransformed into glucose. The liver is a storage organ for fats and takes up food fat as well as fat from depots (in starvation) and as it seems, at least in part, prepares them so that they can be further used in the animal body.

We are not clear as to what extent an assimilation of products of protein digestion takes place in the liver. The subject will be discussed in detail under absorption in Chapter VIII. It is claimed that the liver can serve as a storage organ for proteins,[1] and it is at least certain that it retains alien protein which is brought to it by the blood.[2] The retention of alien protein stands probably in close relationship to the ability of the liver to take up and retain foreign substances as a group from the blood. This is not only true for different metals but also, as shown by several investigators,[3] alkaloids which perhaps are also partly decomposed in the liver. Toxins are also withheld by the liver and hence this organ has a protective action against poisons.

The formation of glycogen from glucose is one of the numerous syntheses occurring in the liver and this is no doubt the one which takes place to the greatest extent. Other syntheses in the liver are, for example,

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[1] See Seitz, Pflüger's Arch., 111 and Asher and Boehm, Zeitschr. f. Biol. 51.

[2] See Reach, Bioch. Zeitschr., 16 and Pacchioni and Carlini, Maly's Jahresb., 39.

[3] Roger, Action du foie sur les poisons (Paris, 1887), which quotes the works of Schiff, Heger and others; also W. N. Woronzow, Maly's Jahresb., 40 and Z. Vamossy, *ibid.*, 40.

the formation of urea or uric acid (in birds) from ammonium salts, the formation of etheral sulphuric acids and conjugated glucuronic acids from the phenols produced in intestinal putrefaction and the recently shown syntheses of amino-acids in the liver. On the other hand a deamidation of amino-acids and purine bodies, hydrolyses, oxidations, reductions and fermentative processes of various kinds occur in the liver. Because of these diverse processes, the results of which we must especially mention the formation of bile as well as the fact that the liver is introduced between the intestine and the general circulation, makes the liver a central organ for metabolism.

Among the numerous chemical processes which take place in the liver there are especially two which give special interest to this organ, namely, the formation of glycogen or the carbohydrate metabolism in the liver, and the formation of bile. For this reason only these two processes will be discussed in this chapter while the others will be discussed in other chapters and in other connection. Before we begin to discuss these two processes a short review of the constituents and the chemical composition of the liver seems to be appropriate.

The reaction of the liver-cells is alkaline toward litmus during life, but becomes acid after death, due to a formation of lactic acid, chiefly fermentation lactic acid and other organic acids (MORISHIMA, MAGNUS-LEVY [1]). A coagulation of the protoplasmic proteins in the cells probably takes place. A positive difference between the proteins of the dead and the living, non-coagulated protoplasm has not been observed.

The *proteins* of the liver were first carefully investigated by PLÓSZ. He found in the watery extract of the liver an *albuminous substance* which coagulates at 45° C. (globulin, HALLIBURTON), also a *globulin* which coagulates at 75° C., a *nucleoalbumin* which coagulates at 70° C., and lastly a protein body which is closely related to the *coagulated albumins* and which is insoluble in dilute acids or alkalies at the ordinary temperature, but dissolves on the application of heat, being converted into an albuminate. HALLIBURTON [2] found two globulins in the liver-cells, one of which coagulates at 68–70° C., and the other at 45–50° C. He also found, besides traces of albumin, a nucleoprotein which possessed 1.45 per cent phosphorus and a coagulation-point of 60° C. POHL has obtained an "organ plasma" by extracting the finely divided liver which had previously been entirely freed from blood by washing with 8 p. m. NaCl solution, in which he was able to detect a globulin having a low coagulation temperature. The very variable phosphorus content

[1] Morishima, Arch. f. exp. Path. u. Pharm., 43; Magnus-Levy, Hofmeister's Beiträge, 2.

[2] Plósz, Pflüger's Arch., 7; Halliburton, Journ. of Physiol., 13, Suppl. 1892.

(0.28–1.3 per cent) of this globulin as well as the insolubility of the precipitates produced by little acid, in an excess of acid, and in neutral salts seem to indicate that we here have a mixture which consists chiefly of nucleoproteins and not of globulins. The almost complete digestibility with pepsin-hydrochloric acid does not controvert this assumption, because, as is known, nucleoproteins may on digestion yield no residue (see Chapter II). Nor can we be positive concerning the nature of the liver-globulin found by DASTRE,[1] having a coagulation temperature of 56°C. The proteins extractable from the liver without modification must be thoroughly investigated.

Besides the above-mentioned proteins, which are very soluble, the liver-cells contain large quantities of difficultly soluble protein bodies (see PLÓSZ). The liver also contains, as first shown by ST. ZALESKI and later substantiated by several other investigators, ferruginous proteins of different kinds.[2]. The chief portion of the protein substances in the liver seems in fact to consist of ferruginous nucleoproteins. On boiling the liver with water, such a nucleoprotein or perhaps several are split, and a solution is obtained containing a nucleic-acid-rich nucleoprotein or a mixture of these which are precipitable by acids. This protein or protein mixture, which has been called *ferratin* by SCHMIEDEBERG,[3] has been studied by WOHLGEMUTH.[4] The quantity of phosphorus was 3.06 per cent. As cleavage products on hydrolysis he found *l*-xylose, or at least a pentose, the four nuclein bases, and also arginine, lysine (and histidine?), tyrosine, leucine, glycocoll, alanine, α-proline, glutamic acid, aspartic acid, phenylalanine, oxyaminosuberic acid, and oxydiaminosebacic acid (see Chapter II). The *l*-xylose depends, no doubt, at least in part, upon the guanylic acid isolated from the liver, by LEVENE and MANDEL,[5] and the finding of adenine among the cleavage products also indicates the presence of a thymonucleic acid. There does not seem to be any doubt that the ferratin, as above stated, is a mixture, and the correctness of this assumption is shown by the recent investigations of SCAFFIDI and SALKOWSKI.[6]

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[1] Pohl, Hofmeister's Beiträge, 7; Dastre, Compt. rend. soc. biolog., 58.

[2] St. Zaleski, Zeitschr. f. physiol. Chem., 10, 486; Woltering, *ibid.*, 21; Spitzer, Pflüger's Arch., 67.

[3] Arch. f. exp. Path. u. Pharm., 33; see also Vay, Zeitschr. f. physiol. Chem., 20.

[4] Wohlgemuth, Zeitschr. f. physiol. Chem., 37, 42, and 44, and Ber. d. d. chem. Gesellsch., 37. See on liver nucleoproteins also Salkowski, Berl. klin. Wochenschr., 1895; Hammarsten, Zeitschr. f. physiol. Chem., 19; Blumenthal, Zeitschr. f. klin. Med., 34.

[5] Bioch. Zeitschr., 10.

[6] Scaffidi, Zeitschr. f. physiol. Chem., 58; Salkowski, *ibid.*, 58.

The yellow or brown pigment of the liver has been little studied. DASTRE and FLORESCO [1] differentiate, in vertebrates and certain invertebrates, between a ferruginous pigment soluble in water, *ferrine*, and a pigment soluble in chloroform and insoluble in water, *chlorochrome*. They have not isolated these pigments in a pure condition. In certain invertebrates chlorophyll originating from the food also occurs in the liver.

The *fat* of the liver occurs partly as very small globules and partly (especially in nursing children and suckling animals, as also after food rich in fat) as rather large fat-drops. The occurrence of a fatty infiltration, i.e., a transportation of fat to the liver, may not only be produced by an excess of fat in the food (NOËL-PATON), but also by a migration from other parts of the body under abnormal conditions, such as poisoning with phosphorus, phlorhizin, and certain other bodies (LEO, LEBEDEFF, ROSENFELD, and others [2]). The fatty infiltration occurring in poisoning, and which is accompanied with degenerative changes in the cells, may cause a diminution in the amount of protein and a rise in the water content. If the amount of fat in the liver is increased by an infiltration, the water decreases correspondingly, while the quantity of the other solids remains little changed. Changes of a kind may occur, so that, because of the antipathy (ROSENFELD, BOTTAZZI[3]) existing between glycogen and fat, a liver rich in fat is habitually poor in glycogen. The reverse occurs after feeding with carbohydrate-rich food, namely, the liver is rich in glycogen and poor in fat.

The composition of the liver-fat seems to vary not only in different animals, but is variable with differing conditions. Thus NOËL-PATON found that the liver-fat in man and several animals was poorer in oleic acid and had a correspondingly higher melting-point than the fat from the subcutaneous connective tissue, while ROSENFELD [4] observed the opposite condition on feeding dogs with mutton-fat.

Several investigators, HARTLEY, LEATHES and MOTTRAM suggested as a difference between the fat of the liver and the connective tissues, the great amount in the first of unsaturated, higher fatty acids. According to HARTLEY [5] the fat of the pig liver contains palmitic acid, stearic

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[1] Arch. de Physiol. (5), **10**.

[2] Noël-Paton, Journ. of Physiol., **19**; Leo, Zeitschr. f. physiol. Chem., **9**; Lebedeff, Pflüger's Arch., **31**; Athanasiu, Pflüger's Arch., **74**; Taylor, Journ. of Exp. Med., **4**; Kraus u. Sommer, Hofmeister's Beiträge, **2**; Rosenfeld, Zeitschr. f. klin. Med., **36**. See also Rosenfeld, Ergebnisse der Physiologie, **1**, Abt. 1, and Berl. klin. Wochenschr. 1904; Schwalbe, Centralbl. f. Physiol., **18**, p. 319; Shibata, Bioch. Zeitschr., **37**.

[3] Arch. Ital. d. Biol., **48** (1908), cited in Bioch. Centralbl., **7**, p. 833.

[4] Cited by Lummert, Pflüger's Arch., **71**. In regard to the liver-fat of children, see Thiemich, Zeitschr. f. physiol. Chem., **26**.

[5] Hartley, Journ. of Physiol., **38**; Leathes and Meyer-Wedell, *ibid.*, **38**; Mottram, *ibid.*, **38**.

acid, and oleic acid which is not identical with the ordinary oleic acid, also linoleic acid and an acid having the formula $C_{20}H_{32}O_2$. A part of these unsaturated fatty acids are contained in the phosphatides but as the unsaturated acids are about one-half of the fatty acids they must also occur in the fats. The abundant occurrence of unsaturated fatty acids is considered by the above-mentioned investigators as the first step in the cleavage of the transportable fat from the fat tissues to the liver and destined for use in the body. There is no doubt that the phosphatides are of great importance for this transformation of the fat.

Phosphatides, which were formerly designated lecithin, and whose quantity is generally calculated as such, also belong to the normal constituents of the liver. The quantity (as lecithin) amounts to over 23.5 p. m. according to NOËL-PATON.[1] In starvation the lecithin, according to NOËL-PATON, forms the greater part of the ethereal extract, while with food rich in fat, on the contrary, it forms the smaller part. In the liver of a healthy dog BASKOFF[2] found 84 p. m. phosphatides (calculated as lecithin) in the dry substance. The phosphatides are undoubtedly of various kinds, but they have not been closely studied. Among others, we have *lecithin* and the so-called *jecorin*. *Cholesterin* is also a constituent of the liver, although only in small quantities, and KONDO[3] finds that cholesterin ester occurs in the liver.

Jecorin was first found by DRECHSEL in the liver of horses, and also in the liver of a dolphin, and later by BALDI in the liver and spleen of other animals, in the muscles and blood of the horse, and in the human brain. It contains sulphur and phosphorus, but its constitution is not positively known. Jecorin dissolves in ether, but is precipitated from this solution by alcohol. It reduces copper oxide, and gives a wine-red coloration with an ammoniacal silver-solution. On boiling with alkali and then cooling it solidifies to a gelatinous mass. MANASSE has detected glucose as osazone in the carbohydrate complex of jecorin.

The statement by BING that jecorin is a combination of lecithin and glucose does not follow from the analyses of jecorin thus far known. Jecorin contains sulphur, even as much as 2.75 per cent, and further the relation of P:N in lecithin is 1:1, while in jecorin it is quite different, 1:2 to 1:6. According to the investigations of BASKOFF the liver jecorin, prepared according to DRECHSEL'S suggestion, and when it is so pure that it is completely soluble in ether, and quantitatively precipitated by alcohol from this solution, is a rather constant compound at least in regard to the N, P and glucose content. BASKOFF found as average 2.55 per cent N, 2.87 per cent P, and about 14 per cent glucose. The relation P:N was nearly 1:2 and therefore jecorin is correspondingly a diaminomonophosphatide.

The variable composition and divergent properties of the jecorin isolated and analyzed by various investigators[4] depends, according to BASKOFF, upon imper-

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[1] l. c. See also Heffter, Arch. f. exp. Path. u. Pharm., **28**.

[2] Zeitschr. f. physiol. Chem., **62**.

[3] Bioch. Zeitschr., **26**.

[4] Drechsel, Ber. d. sächs. Gesellsch. d. Wissensch., 1886, p. 44, and Zeitsch. f. Biologie, **23**; Baldi, Arch. f. (Anat. u.) Physiol., 1887, Suppl., 100; Manasse, Zeitschr. f. physiol. Chem., **20**; Bing, Centralbl. f. Physiol., **12**, and Skand. Arch. f. Physiol., **9**;

fect purification. His investigations do not give any explanation for the quantity of sulphur and it is very probable that jecorin is only a mixture of several bodies among which a sulphurized and a phosphorized substance occurs. According to BASKOFF it is very probable that the jecorin is a decomposition product of lecithin (or other phosphatides).

Another phosphatide, which does not reduce directly or after boiling with acid, has been called *heparphosphatide* by BASKOFF. In certain respects this body is similar to cuorin, and the relation P:N = 1.45:1, although it was not pure.

Among the *extractive substances* besides *glycogen*, which will be treated later, rather large quantities of the *purine bases* occur. KOSSEL[1] found in 1000 parts of the dried substance 1.97 p. m. *guanine*, 1.34 p. m. *hypoxanthine*, and 1.21 p. m. *xanthine*. *Adenine* is also contained in the liver. In addition there are found *urea* and *uric acid* (especially in birds), and indeed in larger quantities than in the blood, *paralactic acid*, *choline*, *leucine*, *taurine*, and *cystine*. In pathological cases *inosite* and *amino-acids* have been detected. The occurrence of *bile-coloring matters* in the liver-cell under normal conditions is doubtful; but in retention of the bile the cells may absorb the coloring-matter and become colored thereby.

A large number of enzymes are found in the liver, such as *catalases*, *oxidases*, *aldehydases* and *hydrolytic enzymes* of various kinds; the *diastase* acting upon glycogen, the *lipases* and the different *proteolytic* enzymes. *Nucleases* and the *nucleic acid splitting enzymes* of different kinds mentioned in Chapter II have been formed in the liver and *deamidases* for amino-acids as well as purine bodies also occur in the liver. The last group of deamidases show a different behavior in regard to their occurrence in different animals and the same is true for the *uric acid forming* and *uric acid destroying* enzymes (Chapter XIV). We must also mention the *arginase* which splits off urea from arginine.

The proteolytic enzymes of the liver are of special interest, especially in regard to the study of the autolysis of this organ. The processes in the liver in phosphorus poisoning and in acute yellow atrophy of the liver are considered as an intravitally increased autolysis. In these cases a softening of the organ takes place, and proteoses, mono- and diamino-acids, and other bodies are produced, which may also be found in the urine, and although they may not all be derived from the liver (NEUBERG and RICHTER), they are at least in part derived from this organ. WAKEMAN has found in phosphorus poisoning that not only is the quantity of nitrogen markedly diminished in the liver (of dogs), but also that the quantity of nitrogen of the hexone bases is diminished, and

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Meinertz, Zeitschr. f. physiol. Chem., **46**; Siegfried and Mark, *ibid.*; Paul Mayer, Bioch. Zeitschr., **1**, and Baskoff, Zeitschr. f. physiol. Chem., **57**, **61**, **62**.

[1] Zeitschr. f. physiol. Chem., 8.

that the part of the protein molecule richer in nitrogen is first removed and eliminated under these conditions. A similar condition has been observed by WELLS in the idiophatic, acute yellow atrophy of the liver. In consideration of the variable results for the diamino-nitrogen even under normal conditions (GLIKIN and A. LOEWY [1]), it is desirable that a greater number of observations be made on this subject. The increased consumption of glycogen under the above-mentioned pathological conditions may also be considered as an increased autolysis, while the claim of certain observers that fat is formed in the autolysis of the liver is, according to SAXL,[2] to be considered only as a more pronounced appearance of the fat previously occurring in the organ.

Besides the above-mentioned organic constituents in the liver we must mention the *glucothionic acid* found by MANDEL and LEVENE,[3] whose chemical individuality is doubted.

The *mineral bodies* of the liver consist of phosphoric acid, potassium, sodium, alkaline earths, and chlorine. The potassium is in excess of the sodium. Iron is a regular constituent of the liver, but it occurs in very variable amounts. BUNGE found 0.01–0.355 p. m. iron in the blood-free liver of young cats and dogs. This was calculated on the liver substance freshly washed with a 1-per cent NaCl solution. Calculated on 10 kilos bodily weight, the iron in the liver amounted to 3.4–80.1 mg. Recent determinations of the quantity of iron in the liver of the rabbit, dog, hedge-hog, pig, and man have been made by GUILLEMONAT and LAPICQUE, and in rabbits by SCAFFIDI. The variation was great in human beings. In men the quantity of iron in the blood-free liver (blood-pigment subtracted in the calculation) was regularly 0.23 p. m., and in women 0.09 p. m. (calculated on the fresh moist organ), and this relation was not changed after the twentieth year. Above 0.5 p. m. is considered as pathological. According to BIELFELD,[4] who worked with another method, an even greater quantity of iron occurs in men.

The quantity of iron in the liver can be increased by drugs containing iron. The quantity of iron may also be increased by an abundant destruction of red blood-corpuscles, which will result from the injection

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[1] Neuberg and Richter, Deutsch. med. Wochenschr., 1904; Wakeman, Zeitschr. f. physiol. Chem., **44**; Wells, Journ. of Exper. Med., **9**; Glikin and Loewy, Bioch. Zeitschr., **10**.

[2] Hofmeister's Beiträge, **10**.

[3] Mandel and Levene, Zeitschr. f. physiol. Chem., **45**.

[4] Bunge, Zeitschr. f. physiol. Chem., **17**, 78; Guillemonat and Lapicque, Compt. rend. de soc. biol., **48**, with Baille, *ibid.*, **68**; and Arch de Physiol. (5) **8**; Biefeld, Hofmeister's Beiträge, **2**; see also Schmey, Zeitschr. f. physiol. Chem., **39**; Scaffidi, *ibid.*, **54**.

of dissolved hæmoglobin, in which process the iron combinations derived from the blood-pigments in other organs, such as the spleen and marrow, also seem to take part.[1] A destruction of blood-pigments, with a splitting off of compounds rich in iron, seems to take place in the liver in the formation of the bile-pigments. Even in invertebrates, which have no hæmoglobin, the so-called liver is rich in iron, from which DASTRE and FLORESCO[2] conclude that the quantity of iron in the liver of invertebrates is entirely independent of the decomposition of the blood-pigment, and in vertebrates it is in part so. According to these authors the liver has, on account of the quantity of iron, a specially important oxidizing function, which they call the "*fonction martiale*" of the liver.

The richness in iron of the liver of new-born animals is of special interest—a condition which was shown by the analyses of ST. ZALESKI, but was especially studied by KRÜGER and MEYER. In oxen and cows they found 0.246–0.276 p. m. iron (calculated on the dry substance), and in the cow-fœtus about ten times as much. The liver-cells of a calf a week old contain about seven times as much iron as the adult animal; the quantity decreases in the first four weeks of life, when it reaches about the same amount as in the adult. LAPICQUE[3] also found that in rabbits the quantity of iron in the liver steadily diminishes from the eighth day to three months after birth, namely, from 10 to 0.4 p. m., calculated on the dry substance. "The fœtal liver-cells bring an abundance of iron in the world to be used up, within a certain time, for a purpose not well known." A part of the iron exists as phosphate, but the greater part is in combination in the ferruginous protein bodies (ST. ZALESKI).

The quantity of calcium oxide in the fresh, moist liver of the horse, ox, and pig, according to TOYONAGA, amounts to 0.148–0.193 p. m., or more than the human liver (0.101 p. m. according to MAGNUS-LEVY). The amount of magnesium oxide was remarkably high, namely, 0.168, 0.198 and 0.158 p. m., in the livers of the horse, ox, and pig, respectively, but considerably less than the human liver in which MAGNUS-LEVY found 0.292 p. m. KRÜGER[4] found the quantity of calcium in the livers of adult cattle and of calves to be respectively 0.71 p. m. and 1.23 p. m. of the dried substance. In the fœtus of the cow it is lower than in calves. During pregnancy the iron and calcium in the fœtus are antagonistic;

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[1] See Lapicque, Compt. Rend., **124**, and Schurig, Arch. f. exp. Path. u. Pharm., **41**.

[2] Arch. de Physiol. (5), **10**.

[3] St. Zaleski, l. c.; Krüger and collaborators, Zeitschr. f. Biologie, **27**; Lapicque, Maly's Jahresber., **20**.

[4] Krüger, Zeitschr. f. Biologie, **31**; Toyonaga, Bull. of the College of Agriculture, Tokio, **6**; A. Magnus-Levy, Bioch. Zeitschr., **24**.

that is, an increase in the quantity of calcium in the liver causes a diminution in the iron, and an increase in the iron causes a decrease in the calcium. Copper seems to be a physiological constituent, and occurs to a considerable extent in Cephalopods (HENZE [1]). Foreign metals, such as lead, zinc, arsenic, and others (also iron), are easily taken up and combined by the liver (SLOWTZOFF, v. ZEYNEK, and others [2]).

v. BIBRA [3] found in the liver of a young man who had suddenly died 762 p. m. water and 238 p. m. solids, consisting of 25 p. m. fat, 152 p. m. protein, gelatin-forming and insoluble substances, and 61 p. m. extractive substances.

MAGNUS-LEVY [4] found in the liver of a healthy suicide 606 p. m. water, 394 p. m. solids among which 212.8 p. m. fat occurred. If the total nitrogen, 27 p. m., is calculated as protein the amount would be approximately 169 p. m.

PROFITLICH [5] found 68.2–75.17 per cent water in the dog liver and 70.76–72.86 per cent in the ox liver. The relation N: C in the fat and glycogen-free dried substance was 1:3.21 in dogs and 1:3.13 in oxen or about the same as in flesh (see Chapter XI).

The quantitative composition of the liver may show great variation, depending upon the kind and amount of the food supplied. The amount of carbohydrate (glycogen) and fat may vary considerably, which is due to the fact that the liver is a storage-organ for these bodies, especially for the glycogen.

Based upon special experiments, SEITZ claims that the liver is a storehouse for protein also. In experiments on hens and ducks which had previously been starved, he found that the liver took up abundant protein on feeding meat, and that its weight as compared with the weight after starvation was doubled or quadrupled. As it is characteristic of storage or reserve bodies that their amount in the storage-organs on feeding with such bodies strongly increases in percentage, it is remarkable in SEITZ's feeding experiments that the percentage of protein in the liver did not increase, but rather diminished slightly. In this case we did not have a higher percentage of protein, but an increase in the weight of the total cell mass of the organ, probably brought about by increased work of the liver due to the protein feeding. The investigations of GRUND [6] have shown that with protein feeding in dogs, the relation P:N in the

[1] Zeitschr. f. physiol. Chem., **33**.
[2] Slowtzoff, Hofmeister's Beiträge, **1**; v. Zeynek, see Centralbl. f. Physiol., **15**.
[3] See v. Gorup-Besanez, Lehrbuch d. physiol. Chem., 4. Aufl., p. 711.
[4] Bioch. Zeitschr., **24**.
[5] Pflüger's Arch., **119**.
[6] Seitz, Pflüger's Arch., **111**; Grund, Zeitschr. f. Biol., **54**.

liver was not essentially changed which speaks against a simple storage of food protein.

Glycogen and its Formation.

Glycogen was first discovered by BERNARD. It is a carbohydrate closely related to the starches or dextrins, with the general formula $m(C_6H_{10}O_5)$. Its molecular weight is unknown, but seems to be very large (GATIN-GRUZEWSKA and v. KNAFFL-LENZ[1]). The largest quantities are found in the liver of adult animals, and smaller quantities in the muscles (BERNARD, NASSE). It is found in very small quantities in nearly all tissues of the animal body. Its occurrence in lymphoid cells, blood, and pus has been mentioned in a previous chapter, and it seems to be a regular constituent of all cells capable of development. Glycogen was first shown to exist in embryonic tissues by BERNARD and KÜHNE, and it seems on the whole to be a constituent of tissues in which a rapid cell formation and cell development are taking place. It is also present in rapidly forming pathological tumors (HOPPE-SEYLER). Some animals, as certain mussels (BIZIO), Tænia and Ascaridæ (WEINLAND[2]), are very rich in glycogen. Glycogen also occurs in the vegetable-kingdom, especially in many fungi.

The quantity of glycogen in the liver, as also in the muscles, depends essentially upon the food. In starvation it disappears almost completely after a short time, but more rapidly in small than in large animals, and it disappears earlier from the liver than from the muscles. As shown by C. VOIT, KÜLZ and especially by PFLÜGER,[3] it never entirely disappears in starvation, as a re-formation of glycogen always takes place. After partaking of food, especially such as is rich in carbohydrates, the liver becomes rich again in glycogen, the greatest increment occurring 14 to 16 hours after eating (KÜLZ). The quantity of liver-glycogen may amount to 120–160 p. m. after partaking of large quantities of carbohydrates, and in dogs which had been especially fed for glycogen SCHÖNDORFF and GATIN-GRUZEWSKA found still higher results, even more than 180 p. m. Ordinarily it is considerably less, namely, 12–30 to 40 p. m. The highest amount of glycogen in the liver thus far observed was 201.6 p. m., found by MANGOLD[4] in the frog. The

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[1] Gatin-Gruzewska, Pflüger's Arch., **103**; v. Knaffl-Lenz, Zeitschr. f. physiol. Chem., **46**.

[2] Zeitschr. f. Biologie, **41**. The extensive literature on glycogen may be found in E. Pflüger, Glykogen, 2. Aufl., Bonn, 1905; and in Cremer, "Physiol. des Glykogens," in Ergebnisse der Physiologie, **1**, Abt. 1. In the following pages we shall refer to these works.

[3] Pflüger's Arch., **119**, which contains the literature.

[4] *Ibid.*, **121**.

shark, whose liver is very rich in fat, even though well nourished, only has comparatively low values for the glycogen in the liver, 9.3–23.8 p. m. (BOTTAZZI [1]). According to CREMER the quantity of glycogen in plants (yeast-cells) is, as in animals, dependent upon the food. He finds that the yeast-cells contain glycogen, which disappears from the cells in the auto-fermentation of the yeast, but reappears on the introduction of the cells into a sugar solution.

The quantity of glycogen of the liver (and also of the muscles) is also dependent upon rest and activity, because during rest, as in hibernation, it increases, and during work it diminishes. KÜLZ has shown that by hard work the quantity of glycogen in the liver (of dogs) is reduced to a minimum in a few hours. The muscle-glycogen does not diminish to the same extent as the liver-glycogen. KÜLZ, ZUNTZ and VOGELIUS, FRENTZEL, and others have been able to render rabbits and frogs nearly glycogen-free by suitable strychnine poisoning. The same result is produced by starvation followed by hard work. According to GATIN-GRUZEWSKA,[2] the liver and muscles in rabbits can be made glycogen-free after 36–40 hours by first starving one day and then injecting adrenalin.

Glycogen forms an amorphous, white, tasteless, and non-odorous powder. When perfectly pure, and by proper alcohol precipitation, it can be obtained as rods or prisms which look like crystals (GATIN-GRUZEWSKA). It gives an opalescent solution with water which, when allowed to evaporate on the water-bath, forms a pellicle over the surface that disappears again on cooling. It is undecided whether we here have a true solution or not. Like other colloids, glycogen in water under the influence of the electric current migrates to the anode, on which it collects (GATIN-GRUZEWSKA). According to BOTTAZZI,[3] who obtained the same results, a little acid or a little alkali modify the results so that the glycogen becomes isoelectric. Its aqueous solution is dextrorotatory, and HUPPERT found it to be $(\alpha)_D = +196.63°$. GATIN-GRUZEWSKA has recently obtained the same result by using a perfectly pure solution of glycogen. A solution of glycogen, especially on the addition of NaCl, is colored wine-red by iodine. It may hold cupric hydroxide in solution in alkaline liquids, but does not reduce it. A solution of glycogen in water is not precipitated by potassium-mercuric iodide and hydrochloric acid, but is precipitated by alcohol (on the addition of NaCl when necessary), or

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[1] Arch. Ital. d. Biol., 48; cited in Bioch. Centralbl., 7, 833.

[2] Compt. Rend., 142.

[3] Bottazzi, Chem. Centralbl. 1909 p. 1423; Bottazzi and d'Errico (Pflüger's Arch., 115) have investigated the viscosity, the electrical conductivity and the freezing-point of glycogen solutions at different concentrations.

ammoniacal basic lead acetate. An aqueous solution of glycogen made alkaline with caustic potash (15 per cent KOH) is completely precipitated by an equal volume of 96 per cent alcohol. Tannic acid also precipitates glycogen. It gives a white granular precipitate of benzoyl-glycogen with benzoyl chloride and caustic soda. Glycogen is completely precipitated by saturating its solution at ordinary temperatures with magnesium or ammonium sulphate. It is not precipitated by sodium chloride, or by half saturation with ammonium sulphate (NASSE, NEUMEISTER, HALLIBURTON, YOUNG[1]). On boiling with dilute caustic potash (1-2 per cent) the glycogen may be more or less changed, especially if it has been previously exposed to the action of acid or to BRÜCKE'S reagent (see below) (PFLÜGER). On boiling with stronger caustic potash (even of 36 per cent) it is not injured (PFLÜGER). By diastatic enzymes glycogen is converted into maltose or glucose, depending upon the nature of the enzyme. It is transformed into glucose by dilute mineral acids. According to TEBB[2] various dextrins appear as intermediary steps in the saccharification of glycogen, depending on whether the hydrolysis is caused by mineral acids or enzymes. The glycogen from various animals and different organs is the same according to PFLÜGER.[3] Nor has it been decided whether all the glycogen in the liver occurs as such or whether it is in part combined with protein (PFLÜGER-NERKING). The investigations of LOESCHCKE[4] have shown that we have no positive reasons for this assumption.

The preparation of pure glycogen (most easily from the liver) is generally performed by the method suggested by BRÜCKE, of which the main points are the following: Immediately after the death of the animal the liver is thrown into boiling water, then finely divided and boiled several times with fresh water. The filtered extract is now sufficiently concentrated, allowed to cool, and the proteins removed by alternately adding potassium-mercuric iodide and hydrochloric acid. The glycogen is precipitated from the filtered liquid by the addition of alcohol until the liquid contains 60 vols. per cent. By repeating this and precipitating the glycogen several times from its alkaline and acetic-acid solution it is purified on the filter by washing first with 60 per cent and then with 95 per cent alcohol, then treating with ether, and drying over sulphuric acid. It is always contaminated with mineral substances. To be able to extract the glycogen from the liver or, especially, from muscles and other tissues completely, which is essential in a quantitative estimation, these parts must first be warmed for two hours with strong caustic potash (30 per cent) on the water-bath. As the glycogen changes in this purifica-

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[1] Young, Journ. of Physiol., **22**, citing the other investigators.
[2] Journ. of Physiol., **22**.
[3] Pflüger's, Arch. **129**.
[4] *Ibid.*, **102**.

tion, as suggested by BRÜCKE, it is better, for quantitative determinations of glycogen, to precipitate it directly from the alkaline solution by alcohol (PFLÜGER [1]).

The quantitative estimation is best performed according to PFLÜGER'S method, which is as follows: The finely divided organ is heated on the water-bath for 2–3 hours in the presence of 30 per cent KOH; after diluting with water and filtering, the glycogen is precipitated with alcohol, and the redissolved glycogen estimated in part by the polariscope and in part as sugar after inversion. One part by weight of sugar equals 0.927 part glycogen. As in the estimation the prescribed directions must be exactly followed, we must refer to the original work of PFLÜGER for the details of the method. Other methods of estimating glycogen, such as those of BRÜCKE-KÜLZ, PAVY, and AUSTIN, are described in PFLÜGER'S Archiv. 96. Also compare the recent works of PFLÜGER.[2]

Numerous investigators have endeavored to determine the origin of glycogen in the animal body. It is positively established by the unanimous observations of many investigators [3] that the varieties of *sugars* and their anhydrides, *dextrins* and *starches*, have the property of increasing the quantity of glycogen in the body. The action of inulin seems to be somewhat uncertain.[4] The statements are questioned in regard to the action of the pentoses. CREMER found that in rabbits and hens various pentoses, such as rhamnose, xylose, and arabinose, have a positive influence on the glycogen formation, and SALKOWSKI obtained the same result on feeding *l*-arabinose. FRENTZEL, on the contrary, found no glycogen formation on feeding xylose to a rabbit which had previously been made glycogen-free by strychnine poisoning, and NEUBERG and WOHLGEMUTH [5] obtained similar negative results on feeding rabbits with *d*- and *r*-arabinose. In general we can for the present accept the view that the pentoses are not direct glycogen formers.

The hexoses, and the carbohydrates derived therefrom, do not all possess the ability of forming or accumulating glycogen to the same extent. Thus C. VOIT [6] and his pupils have shown that glucose has a more powerful action than cane-sugar, while milk-sugar is less active (in rabbits and hens) than glucose, fructose, cane-sugar, or maltose.

The following substances when introduced into the body also increase the quantity of glycogen in the liver: *Glycerin, gelatin, arbutin*, and likewise, accord-

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[1] See also the method suggested by Gautier, Compt. Rend., **129**.

[2] Pflüger's Arch., **103, 104, 121** and especially **129**.

[3] In reference to the literature on this subject, see E. Külz, Pflüger's Arch., **24**, and Ludwig, Festschrift, 1891; also the cited works of Pflüger and Cremer, foot-note 2, p. 390.

[4] See Miura, Zeitschr. f. Biologie, **32**, and Nakaseko, Amer. Journ. of Physiol., **4**.

[5] Salkowski, Zeitschr. f. physiol. Chem., **32**; Neuberg and Wohlgemuth, *ibid.*, **35**. See also Pflüger, l. c., and Cremer, l. c.

[6] Zeitschr. f. Biologie, **28**.

ing to the investigations of KÜLZ, *erythrite*, *quercite*, *dulcite*, *mannite*, *inosite*, *ethylene* and *propylene glycol*, *glucuronic anhydride*, *saccharic acid*, *mucic acid*, *sodium tartrate*, *saccharin*, *isosaccharin*, and *urea*. *Ammonium carbonate*, *glycocoll*, and *asparagine* may similarly, according to RÖHMANN, cause an increase in the amount of glycogen in the liver. NEBELTHAU finds that other ammonium salts and some of the amides, as well as certain *narcotics*, *hypnotics*, and *antipyretics*, produce an increase in the glycogen of the liver. This action of the antipyretics (especially antipyrine) had been shown by LÉPINE and PORTERET.[1]

The fats, according to BOUCHARD and DESGREZ, increase the glycogen content of the muscles but not of the liver, while COUVREUR believes that the glycogen is increased at the expense of the fat in the silkworm larva, but these statements have been shown to be incorrect by the recent investigations of KOTAKE and SERA.[2] In general it is believed that fat does not increase the amount of glycogen in the liver or in the animal body, although a carbohydrate formation from glycerin, but not a glycogen formation, is probable.

The question whether the proteins have the ability to increase the glycogen content of the liver or the animal body has been long disputed. The feeding experiments with meat or with pure proteins by older experimenters, such as NAUNYN, v. MERING and E. KÜLZ seem to show an ability. But the proof of these investigations has been strongly disputed by PFLÜGER and later investigations of SCHÖNDORFF, BLUMENTHAL and WOHLGEMUTH, as also those of BENDIX and STOOKEY[3] yield contradictory results. These investigations have really only historical interest, since now a carbohydrate formation as well as a glycogen formation from proteins have been positively observed.

If the question is raised as to the action of the various bodies on the accumulation of glycogen in the liver, it must be recalled that a formation of glycogen takes place in this organ, as well as a consumption of the same. An accumulation of glycogen may be caused by an increased formation of glycogen, but also by a diminished consumption, or by both.

It is not known how the various bodies above mentioned act in this regard. Certain of them probably have a retarding action on the transformation of glycogen in the liver, while others perhaps are more combustible, and in this way protect the glycogen. Some probably excite the liver-cells to a more active glycogen formation, while others yield material from which the glycogen is formed, and are *glycogen-formers*

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[1] Röhmann, Pflüger's Arch., **39**; Nebelthau, Zeitschr. f. Biologie, **28**; Lépine and Porteret, Compt. Rend., **107**.

[2] Bouchard et Desgrez, Compt. Rend., **130**; Couvreur, Compt. rend. de soc. biol., **47**; Kotake and Sera Zeitschr. f. physiol. Chem., **62**.

[3] See the work on glycogen by Pflüger and also Schöndorff, Pflüger's Arch., **82** and **88**; Blumenthal and Wohlgemuth, Berl. klin. Wochenschr., 1901; Bendix, Zeitschr. f. physiol. Chem., **32** and **34**; Stookey, Amer. Journ. of Physiol., **9**.

in the strictest sense of the word. The knowledge of these last-mentioned bodies is of the greatest importance in the question as to the origin of glycogen in the animal body, and the chief interest attaches to the question: To what extent are the two chief groups of food, the proteins and carbohydrates, glycogen-formers?

The great importance of the carbohydrates in the formation of glycogen has given rise to the opinion that the glycogen in the liver is produced from sugar by a synthesis in which water separates with the formation of an anhydride (LUCHSINGER and others). This theory (*anhydride theory*) has found opponents because it neither explains the formation of glycogen from such bodies as proteins, carbohydrates, gelatin, and others, nor the circumstance that the glycogen is always the same, independent of the properties of the carbohydrate introduced, whether it is dextrogyrate or levogyrate. This last circumstance does not now present any special difficulty, since we know that the simple sugars can easily be transformed into each other. It was formerly the opinion of many investigators that all glycogen is formed from protein, and that this splits into two parts, one containing nitrogen and the other being free from nitrogen; the latter is the glycogen. According to these views, the carbohydrates act only in that they spare the protein and the glycogen produced therefrom (*sparing theory* of WEISS, WOLFFBERG, and others [1]).

In opposition to this theory C. and E. VOIT and their pupils have shown that the carbohydrates are " true " glycogen-formers. After partaking of large quantities of carbohydrates, the amount of glycogen stored up in the body is sometimes so great that it cannot be covered by the proteins decomposed during the same time, and in these cases a glycogen formation from the carbohydrates must be admitted. According to CREMER only the fermentable sugars of the six carbon series or their di- and polysaccharides are *true glycogen-formers*. For the present, only glucose, fructose, and to a much less degree galactose (WEINLAND [2]), and perhaps also *d*-mannose (CREMER) are designated as true glycogen-formers. Other monosaccharides may indeed, according to CREMER, influence the formation of glycogen, but they are not converted into glycogen, and hence are called only *pseudoglycogen-formers*.

The poly- and disaccharides may, after a cleavage into the corresponding fermentable monosaccharides, serve as glycogen-formers. This is true for at least cane-sugar and milk-sugar, which must first

[1] In regard to these two theories, see especially Wolffberg, Zeitschr. f. Biologie, **16**.

[2] E. Voit, Zeitschr. f. Biologie, **25**, 543, and C. Voit, *ibid.*, **28**. See also Kausch and Socin, Arch. f. exp. Path. u. Pharm., **31**; Weinland, Zeitschr. f. Biologie, **40** and **38**; Cremer, *ibid.*, **42**, and Ergebnisse der Physiol., 1.

be inverted in the intestine. These two varieties of sugar, therefore, cannot, like glucose and fructose, serve as glycogen-formers after subcutaneous injection, but reappear almost entirely in the urine (DASTRE, FR. VOIT). Maltose, which is inverted by an enzyme present in the blood, passes only to a slight extent into the urine (DASTRE and BOURQUELOT and others), and it can, like the monosaccharides, even after subcutaneous injection, be used in the formation of glycogen (FR. VOIT).[1] Of the disaccharides the maltose and the cane-sugar are strong glycogen-formers while milk-sugar has only a weak action.

The ability of the liver to form glycogen from monosaccharides has also been shown by K. GRUBE in a very interesting and direct manner, by perfusion experiments with solutions of various carbohydrates. In these perfusion experiments on tortoise livers, glucose produced an abundant glycogen formation, while with fructose and galactose it was less abundant. Pentoses, disaccharides, casein and amino-acids (glycocoll, alanine and leucine) were inactive while on the contrary glycerin and also formaldehyde acted as glycogen-formers. The formation of glycogen from formaldehyde is disputed by SCHÖNDORFF and GRUBE.[2]

After PAVY[3] first showed the occurrence of carbohydrate groups in ovalbumin, other investigators were able to split off glucosamine from this and other protein substances (see Chapter II), and the question arose whether the amino-sugar could serve in the formation of glycogen. The investigations carried out in this direction by FABIAN, FRÄNKEL and OFFER, CATHCART and BIAL, have shown that the glucosamine introduced into the organism is in part eliminated unchanged in the urine and has no glycogen-forming action. No definite conclusions can be drawn from this on the behavior of the carbohydrate groups, which exist not as free groups but combined with the protein molecules. The investigations of FORSCHBACH on the behavior of glucosamine chained to an acid-group in an amide-like combination, as well as the investigations of KURT MEYER and STOLTE,[4] have yielded no proofs for the theory that glycogen is formed from glucosamine.

Whether or not, or to what extent, the glucoproteins by their glucosamine component take part in the sugar or glycogen formation in the animal

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[1] Dastre, Arch. de Physiol. (5) **3**, 1891; Dastre and Bourquelot, Compt. Rend., **98**; Fritz Voit, Verhandl. d. Gesellsch. f. Morph. u. Physiol. in München, 1896, and Deutsch. Arch. f. klin. Med., **58**. In regard to the glycogen formation after intravenous injection of sugar see FREUND and POPPER, Bioch. Zeitschr., **41**.

[2] Pflüger's Arch., **138**; Grube, *ibid.*, **118**, **121**, **122**, **126** and **139**.

[3] The Physiology of the Carbohydrates, London, 1894.

[4] Fabian, Zeitschr. f. physiol. Chem., **27**; Fränkel and Offer, Centralbl. f. Physiol., **13**; Cathcart, Zeitschr. f. physiol. Chem., **39**; Bial, Berl. klin. Wochenschr., **1905**; Forschbach, Hofmeister's Beiträge, 8; Meyer, *ibid.*, **9**; Stolte, *ibid.*, **11**.

body is difficult to answer for the present, as but little is known of the quantity of these substances in the body, and our knowledge of the amount of carbohydrate which can be split off from the various protein substances is also very meager.

If the proteins are to be counted, and this is in agreement with the generally accepted view, among those bodies which increase the glycogen of the body, then we must ask the question: Do the proteins act only indirectly as pseudoglycogen-formers, or are they direct glycogen-formers which can serve as material for the formation of glycogen or sugar? This question stands in close relation to the sugar formation and sugar elimination in the various forms of glycosuria, and will be best discussed below in connection with the question of diabetes.

Glycogen is a reserve-food deposited, in the liver and which, like other carbohydrates can be transformed into fat, and it is generally admitted that such a fat formation from glycogen also takes place in the liver. There is no doubt that the glycogen deposited in the liver is formed in the liver-cells from the sugar; but where does the glycogen existing in the other organs, such as the muscles, originate? Is the glycogen of the muscles formed on the spot or is it transmitted to the muscles by the blood? These questions cannot at present be answered with certainty, and the investigations on this subject by different experimenters have given varying results. The experiments of KÜLZ,[1] in which he studied the glycogen formation by passing blood containing cane-sugar through the muscle, have led to no conclusive results, while the perfusion experiments of HATCHER and WOLFF with glucose seem to indicate a glycogen formation from sugar in the muscles. The investigations of DE FILIPPI[2] on dogs with so-called Eck's fistula also show a glycogen formation from sugar in the muscles. In the Eck fistula operation the portal vein is ligated near the liver. hilus and sewed to the inferior vena cava and an opening established between the two veins so that the portal blood flows directly into the vena cava without passing through the liver. In well-nourished animals, operated upon in this manner, the livers had the same properties as those from starving animals, while, on the contrary, the muscles contained quantities of glycogen which corresponded to those found in a normal over-fed dog.

If it be true that the blood and lymph contain a diastatic enzyme which transforms glycogen into sugar, and also that the glycogen regularly occurs in the form-elements and is not dissolved in the fluids, it seems probable that the glycogen in solution is not transmitted by the blood to

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[1] See Minkowski and Laves, Arch. f. exp. Path. u. Pharm., **23**; Külz, Zeitschr. f. Biologie, **27**; Hatcher and Wolff, Journ. of Biol. Chem., **3**.

[2] Zeitschr. f. Biol., **49** and **50**.

the organs, but perhaps more likely, if the leucocytes do not act as carriers, it is formed on the spot from the sugar.[1] The glycogen formation seems to be a general function of the cells. In adults, the liver, which is very rich in cells, has the property, on account of its anatomical position, of transforming large quantities of sugar into glycogen.

This glycogen, which is deposited in the liver as reserve-food, in order that it can be useful to the body, must at least in greater part be transformed into sugar and supplied to the various organs by the blood. The question now arises whether there is any foundation for the statement that the liver glycogen is transformed into sugar.

As first shown by BERNARD and redemonstrated by many investigators, the glycogen in a dead liver is gradually changed into sugar, and this sugar formation is caused, as BERNARD supposed and then shown by numerous investigators by a diastatic enzyme whose relation to the diastatic enzyme of the blood is not quite clear.[2]

This post-mortem sugar formation led BERNARD to the assumption of the formation of sugar from glycogen in the liver during life. BERNARD suggested the following arguments for this theory: The liver always contains some sugar under physiological conditions, and the blood from the hepatic vein is always somewhat richer in sugar than the blood from the portal vein. BERNARD'S views found in SEEGEN an active supporter, as he tried to show by numerous experiments the physiological sugar content of the liver as well as the high sugar content of the blood of the liver veins. On the other hand the correctness of the observations of BERNARD and SEEGEN is disputed by many investigators such as PAVY, RITTER, SCHIFF, EULENBERG, LUSSANA, MOSSE, N. ZUNTZ and others,[3] and in regard to the sugar content in the two kinds of blood we have come to the general conclusion that when only the stasis and other disturbing influences of the operation are prevented, the blood of the liver veins, if at all, is only slightly richer in sugar than the blood of the portal vein.[4]

The circumstance that the blood-sugar rapidly sinks to $\frac{1}{2}$–$\frac{1}{3}$ of its original quantity, or even disappears when the liver is cut out of the circulation, indicates a vital formation of sugar in the liver (SEEGEN, BOCK and HOFFMANN, KAUFMANN, PAVY and others). In geese whose

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[1] See Dastre, Compt. rend. de soc. biol., **47**, 280, and Kaufmann, *ibid.*, 316.

[2] Röhmann, Verh. d. Ges. deutsch. Naturf. u. Aerzte. Breslau, 1903; Borchardt, Pflüger's Arch., **100**; Zegla, Bioch. Zeitschr., **16**; E. Starkenstein, *ibid.*, **24**.

[3] In regard to the literature on sugar formation in the liver see Bernard, Leçons sur le diabète, Paris, 1877; Seegen, Die Zuckerbildung im Tierkörper, 2. Aufl. Berlin, 1900; M. Bial, Pflüger's Arch., **55**, 434.

[4] Seegen, Die Zuckerbildung, etc., and Centralbl. f. Physiol., **10**, 497 and 822; Zuntz, *ibid.*, 561; Mosse, Pflüger's Arch., **63**; Bing, Skand. Arch. f. Physiol., **9**.

livers were removed from the circulation, MINKOWSKI found no sugar in the blood after a few hours. On removing the liver from the circulation by tying all the vessels to and from the organ, the quantity of sugar in the blood is not increased (SCHENCK [1]). An important proof of the possibility of a vital formation of sugar from the liver glycogen lies in the fact that we shall learn below of certain poisons and operative changes which may cause an abundant elimination of sugar, but only when the liver contains glycogen.

A vital formation of sugar from the liver glycogen is now generally accepted. Most investigators consider this as an enzymotic transformation of the glycogen by means of the liver diastase, while certain investigators such as DASTRE, NOËL-PATON, E. CAVAZZANI, MCGUIGAN and BROOKS [2] and others explain it by a special activity of the protoplasm. BANG [3] has studied the formation of sugar in frogs' livers, which had not appreciably changed in weight in a RINGER'S solution which was isotonic with the frog blood and which correspondingly had retained their vital properties. This sugar formation does not depend upon a protoplasmic activity but is of an enzymotic nature. It is caused by a diastase, which in Rana esculenta occur in great part in a latent, inactive form due to the inhibitory action of the liver lipoids. Common salt is especially important as an activator for this enzyme. The surviving frog liver is stimulated to a strong sugar production by adrenalin, and this sugar formation is also of an enzymotic nature. The action of the adrenalin consists in an activation of the liver diastase, brought about in various ways.

The relation of the sugar eliminated in the urine under certain conditions, such as in diabetes mellitus, certain intoxications, lesions of the nervous system, etc., to the glycogen of the liver is also an important question.

It does not enter into the plan and scope of this book to discuss in detail the various views in regard to glycosuria and diabetes. The appearance of glucose in the urine is a symptom which may have essentially different causes, depending upon different circumstances. Only a few of the most important points will be mentioned.

The blood always contains about the average of 1 p. m., while the urine has in it at most only traces of glucose. When the quantity of

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[1] Seegen, Bock, and Hoffmann, see Seegen, l. c.; Kaufmann, Arch. de Physiol. (5), **8**; Tangl and Harley, Pflüger's Arch., **61**; Pavy, Journ. of Physiol., **29**, Minkowski, Arch. f. exp. Path. u. Pharm., **21**; Schenck, Pflüger's Arch., **57**.

[2] See Dastre, Noël-Paton, Cavazzani and their work cited in Pick, Hofmeister's Beiträge, **8**, and McGuigan and Brooks, Amer. Journ. of Physiol., **18**; R. G. Pearce, *ibid.*, **25**.

[3] Bioch. Zeitschr., **49**.

sugar in the blood rises above this average, sugar passes into the urine, sometimes even with slight rise and in other cases with stronger rise. The kidneys have the property to a certain extent of preventing the passage of blood-sugar into the urine; and it follows from this that an elimination of sugar in the urine may be caused partly by a reduction or suppression of this above-mentioned activity, and partly also by an abnormal increase of the quantity of sugar in the blood.

The first seems, according to v. MERING and MINKOWSKI, and others to be the case in phlorhizin diabetes. v. MERING found that a strong glycosuria appears in man and animals on the administration of the glucoside phlorhizin. The sugar eliminated is not derived from the glucoside alone. It is formed in the animal body, and in fact from the carbohydrates, or as generally admitted on prolonged starvation, from the protein substances of the body (LUSK). The quantity of sugar in the blood is not increased, but rather diminished, in phlorhizin diabetes (MINKOWSKI), which does not indicate increase in the sugar production but rather an increased excretion of the sugar by the kidneys. The fact that after extirpation of the kidney in phlorhizin diabetes no rise in the blood-sugar is observed, and that after the injection of phlorhizin in the renal artery of one side the urine secreted by this kidney contains sugar sooner and more abundantly than the urine from the other kidney (ZUNTZ), tends to favor this view. The experiments especially performed by PAVY, BRODIE, and SIAU upon blood containing phlorhizin and surviving kidneys also indicate the same, namely, that the phlorhizin acts upon the kidneys and the researches of ERLANDSEN also lead to the same conclusion. He found that on combining the phlorhizin action with bleeding that the glycosuria was increased while after bleeding alone without phlorhizin poisoning the hyperglycæmia was absent. While v. MERING and others believe in an increased permeability of the kidneys for sugar, produced by the phlorhizin LÉPINE [1] is of the view that the phlorhizin causes a formation of glucose from the virtual sugar in the kidneys. PAVY is, on the contrary, of the opinion that the kidneys, under the influence of the phlorhizin, split off sugar from a substance

[1] In regard to the literature on phlorhizin diabetes see v. Mering, Zeitschr. f. klin. Med., **14** and **16**; Minkowski, Arch. f. exp. Path. u. Pharm., **31**; Moritz and Prausnitz, Zeitschr. f. Biologie, **27** and **29**; Külz and Wright, *ibid.*, **27**, 181; Cremer and Richter, *ibid.*, **28** and **29**; Contejean, Compt. rend. de soc. biol., **48**; Lusk, Zeitschr. f. Biologie, **36** and **42**; Levene, Journal of Physiol., **17**; Pavy, *ibid.*, **20**, and with Brodie and Siau, **29**; Arteaga, Amer. Journ. of Physiol., **6**; O. Loewi, Arch. f. exp. Path. u. Pharm., **47**; N. Zuntz, Arch. f. (Anat. u.) Physiol., 1895; Stiles and Lusk, Amer. Journ. of Physiol., **10**; Lusk, *ibid.*, **22**; Cremer, Ergebnisse der Physiol., **1**, Abt. 1; Erlandsen, Bioch. Zeitschr., **23** and **24**; Lépine, Compt. rend. soc. biol., **68**; Lusk, Ergebnisse der Physiol., Bd. **12**, 315–392, and the monographs upon diabetes.

circulating in the blood, perhaps from a protein with loosely combined carbohydrate groups.

GRUBE from experiments upon the surviving tortoise liver has made the suggestion that it is not the kidneys which are first attacked by the phlorhizin action in phlorhizin glycosuria but the liver. Important experimental evidence against this view has been raised by SCHÖNDORFF and SUCKROW.[1]

Another form of glycosuria which according to certain investigators is to be connected with a changed permeability of the kidneys (UNDERHILL and CLOSSON) is the glycosuria first observed by BOCK and HOFFMANN after the intravascular injection of large quantities of a 1-per cent salt solution, which is also of great interest because, as shown by MARTIN FISCHER,[2] it can be again arrested by an injection of a salt solution containing $CaCl_2$. There are investigators who attempt to connect this glycosuria with the adrenals and a hyperglycæmia.

With the exception of these two forms of glycosuria, the phlorhizin diabetes and the salt-glycosuria, and also the glycosuria produced by certain kidney poisons, all other forms of glycosuria or diabetes, as far as known at present, depend on a *hyperglycæmia*.

A hyperglycæmia may be caused in various ways. It may be caused, for example, by the introduction of more sugar than the body can destroy.

The ability of the animal body to assimilate the different varieties of sugar has naturally a limit. If too much sugar is introduced into the intestinal tract at one time, so that the so-called assimilation limit (see Chapter VIII, on absorption) is overreached, then the excess of absorbed sugar passes into the urine. This form of glycosuria is called *alimentary glycosuria*,[3] and is caused by the passage of more sugar into the blood than the liver and other organs can destroy.

As the liver cannot transform into glycogen all the sugar which comes to it in these, to a certain extent physiological, alimentary glycosurias, it is possible that a glycosuria may also be produced under pathological conditions, even by a moderate amount of carbohydrate (100 grams glucose), which a healthy person could overcome. This is true, among other cases, in various affections of the cerebral system and in certain chronic poisonings. Certain observers include the lighter forms of

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[1] Grube, Pflüger's Arch., **128**; Schöndorff and Suckrow, *ibid.*, **138**. See also the opposed view of Underhill, Journ. of biol. Chem., **13**.

[2] Bock and Hoffmann, Arch. f. (Anat. u.) Physiol., 1871; M. Fischer, University of California publications Physiol., 1903 and 1904, and Pflüger's Arch., **106** and **109**; Underhill and Closson, Amer. Journ. of Physiol., **15**, and Journ. of Biol. Chem., **4**.

[3] In regard to alimentary glycosuria see Moritz, Arch. f. klin. Med., **46**, which also contains the earlier literature; B. Rosenberg, Ueber das Vorkommen der alimentären Glykosurie, etc. (Inaug.-Dissert. Berlin, 1897); van Oondt, Münch. med. Wochenschr., 1898; v. Noorden, Die Zuckerkrankheit, 3. Aufl., 1901.

diabetes, where the sugar disappears from the urine when the carbohydrates are cut off as much as possible from the food, in this class of glycosuria.

A hyperglycæmia which passes into a glycosuria may also be brought about by an excessive or sudden formation of sugar from the glycogen and other substances within the animal body.

To this group of glycosurias belongs, it seems, the *adrenalin glycosuria*, in which an increased mobilization of the carbohydrate occurs, especially the liver glycogen. Several circumstances indicate this origin of the sugar. Thus, after adrenalin injection the glycogen disappears from the liver and, according to MICHAUD,[1] adrenalin is without action in dogs with Eck fistula. The activity of the adrenalin in starving animals whose livers are very poor in glycogen speaks for the possibility that the sugar also may in part have another origin than that from the liver glycogen.

Adrenalin glycosuria takes, to a certain degree, a central position and as such a glycosuria we consider also several other forms of glycosuria caused by hyperglycæmia. This is for example the case with the glycosuria after BERNARD'S *sugar puncture* or *piqûre*. That the glycosuria produced after *piqûre* is due to an increased transformation of the glycogen, follows from the fact that no glycosuria appears, under the abovementioned circumstances, when the liver has been previously made free from glycogen by starvation or other means. The close relation of this form of hyperglycæmia and glycosuria to the adrenals follows from the fact that the sugar puncture is without action after the extirpation of the two adrenals. In rats, SCHWARZ found, after such a double extirpation of the adrenals, that the liver was glycogen free and he considers this lack of glycogen as the cause for the inaction of the piqûre under these conditions. According to KAHN and STARKENSTEIN [2] the conditions must be different, as they found in rabbits who remained alive a year after the total extirpation of the adrenals, that the liver had a normal amount of glycogen and that the sugar puncture nevertheless was without action. Adrenalin caused glycosuria in such animals.

It is generally admitted that the stimulation which the sugar center in the fourth ventricle exerts, through the sympathetic nerve reaches to the adrenals and causes a secretion of adrenalin, which increases the sugar formation. Certain circumstances, for example, that a glycosuria can be brought about in starving animals, in which the piqûre is without action, by adrenalin, make the mechanism of this glycosuria somewhat uncer-

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[1] Verhandl. d. deutsch. Kongr. f. inn. Med. Wiesbaden, 1911.

[2] Schwarz, Pflüger's Arch., **134**; Kahn and Starkenstein, *ibid.*, **139**; Kahn, *ibid.*, **140**; Starkenstein, Arch. f. exp. Path. u. Therap., **10**.

tain. Under all circumstances the sugar puncture glycosuria stands in close relation to the adrenals and is generally considered as an adrenalin-glycosuria. The same is true for the glycosuria after splanchnic stimulation and probably for several other forms of glycosuria. In the glycosuria produced by stimulation of the central vagus, according to BANG, LJUNGDAHL and BOHM,[1] the hyperglycæmia (in rabbits) depends upon an increased destruction of the glycogen of the muscles and not of the liver.

Many investigators consider the glycosuria appearing after the occurrence of *dyspnœ*,[2] produced in various ways, and also after certain *poisons* such as carbon monoxide, curare, ether, chloroform, strychnine, morphine, piperidin and others as adrenalin glycosurias. That also in many of such cases the glycosuria is brought about by an increased glycogen destruction is not doubted. In certain cases, as in carbon monoxide poisoning, a formation of sugar has been claimed from protein, because STRAUB and ROSENSTEIN[3] found that this glycosuria only occurred in those animals that had a sufficient quantity of protein at their disposal. Protein starvation and simultaneous abundant carbohydrate supply cause a disappearance of this glycosuria.

A hyperglycæmia and glycosuria may also be caused by a decreased ability of the animal to consume or to utilize the sugar or to transform it into glycogen. In this case the sugar must accumulate in the blood, and the formation of severe cases of diabetes mellitus is now generally explained by this process.

The inability of diabetics to destroy or consume the sugar does not seem to be connected with any decrease in the oxidative energy of the cells. The oxidative processes are not generally diminished in diabetes (SCHULTZEN, NENCKI and SIEBER), and this has recently been substantiated by BAUMGARTEN.[4] This latter investigator made experiments with several bodies which on account of their aldehyde nature were closely related to sugar or were cleavage or oxidation products of it, namely, glucuronic acid, *d*-gluconic acid, *d*-saccharic acid, glucosamine,

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[1] Hofmeister's Bieträge, **10**.

[2] On the importance of the oxygen and the carbon dioxide content of the blood for the non-appearance or appearance of glycosuria see Underhill, Journ. of biol. Chem., **1**; Penzoldt and Fleischer, Virchow's Arch., **87**; Sauer, Pflüger's Arch., **49**, 425, 426; Macleod, Amer. Journ. of Physiol., **19**, with Briggs, Cleveland Med. Journ., 1907; Eddie, Bioch. Journ., **1**, with Moore and Roaf, *ibid.*, **5**; Henderson and Underhill, Amer. Journ. of Physiol., **28**.

[3] Straub, Arch. f. exp. Path. u. Pharm., **38**; Rosenstein, *ibid.*, **40**.

[4] Schultzen, Berl. klin. Wochenschr., 1872; Nencki and Sieber, Journ. f. prakt. Chem. (N. F.), **26**, 35; Baumgarten, "Ein Beitrag zur Zenntniss des Diabetes mellitus," Habilitationschrift, also Zeitschr. f. exp. Path. u. Therap., **2**, 1905.

mucic acid, and others, and he found that diabetics destroyed or burned these bodies to the same extent as healthy individuals. Besides this it must be remarked that the two varieties of sugar, glucose and fructose, which are oxidized with the same readiness, act differently in diabetics. According to KÜLZ and other investigators fructose is, contrary to glucose, utilized to a great extent in the organism, but this in man is, not always the case or at least to a less extent than in certain animals. In animals with pancreas diabetes (see below) fructose [1] may cause a deposition of glycogen in the liver while with glucose this does not occur. The combustion of protein and fat takes place as in healthy subjects, and the fat is completely burned into carbon dioxide and water. In this diabetes the ability of the cells to utilize the glucose suffers diminution, and the explanation of this has been sought in the fact that the glucose is not previously split before combustion.

The variation in the respiratory quotient, i.e., the relation $\frac{CO_2}{O}$, seems to show an insufficiency of the glucose combustion in the tissues in diabetes. As will be thoroughly explained in a subsequent chapter, this quotient is greater the more carbohydrates are burned in the body, and it is correspondingly smaller when protein and fat are chiefly burned. The investigations of LEO, HANRIOT, WEINTRAUD and LAVES,[2] and others have shown that in severe cases of diabetes, in the starving condition, the low quotient is not raised after partaking of glucose, as in healthy individuals, but that it is raised after feeding fructose, which is also of value to diabetics.

The poverty of the organs and tissues of diabetics in glycogen indicates that the glycogen in them is more abundantly transformed into sugar. From what has been said above in regard to the different behavior of fructose and glucose in the glycogen formation in diabetes, indicates that in diabetes, also an inability of the body to transform glucose into glycogen exists and that the lack of glycogen may come about in this way.

Indeed it has been suggested that a preliminary transformation of glucose into glycogen is necessary before it can be burned in the animal body. This assumption is without foundation, at least for the glycogen formation in the liver, as the animal body as is shown with experiments on dogs, can assimilate and burn considerable quantities of carbohydrates even after the liver is excluded (WEHRLE, VERZÁR [3]). The admitted

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[1] Külz, Beiträge zur Path. u. Therap. des Diabetes mellitus (Marburg, 1874), **1**; Weintraud and Laves, Zeitschr. f. physiol. Chem., **19**; Haycraft, *ibid.*; Minkowski, Arch. f. exp. Path. u. Pharm., **31**.

[2] See v. Noorden, Die Zuckerkrankheit, 3. Aufl., 1901.

[3] Wehrle, Bioch. Zeitschr., **34**; Verzár, *ibid.*, **34**.

ability of the liver in diabetes to use fructose and not glucose in the formation of glycogen is, according to E. NEUBAUER,[1] not characteristic for diabetes, because it also occurs in phosphorus poisoning. Whether the different behavior of the two kinds of sugar actually depends upon a diminished ability of the liver in diabetes to form glycogen from glucose or to another unknown circumstance has not been sufficiently proved. In experiments on tortoise livers, by perfusion of RINGER'S solution containing sugar, NISHI [2] found that the livers of diabetic animals formed as much glycogen as the livers of normal animals. These results, which cannot be applied to other animals, require at least further investigation.

The relation of the pancreas to diabetic glycosuria is of the greatest importance for its proper understanding.

The investigations of MINKOWSKI, v. MERING, DOMINICIS, and later of many other investigators,[3] show that a true diabetes of a severe kind is caused by the total or almost total extirpation of the pancreas of many animals, especially dogs. As in man in severe forms of diabetes, so also in dogs with pancreatic diabetes, an abundant elimination of sugar takes place even on the complete exclusion of carbohydrates from the food.

Artificial *pancreas diabetes* may indeed also in other respects present the same picture as diabetes in man, but there exist important differences between these two.[4] It is generally accepted that in pancreas diabetes a diminished consumption exists, i.e., diminished utilization, which does not exclude an increased sugar formation from other bodies not carbohydrates.

Many important observations show that a close relation exists between the liver and pancreas diabetes. PFLÜGER has also especially shown that in diabetes produced by SANDMEYER'S method (partial extirpation with subsequent destruction of the remains of the gland in the abdominal cavity, when the animal remains alive for a longer time than after total extirpation) the liver does not lose weight, although the total weight of the animal diminishes greatly, while in starvation without diabetes

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[1] Arch. f. exp. Path. u. Pharm., **61**.

[2] *Ibid.*, **62**.

[3] See Minkowski, Untersuchungen über Diabetes mellitus nach Exstirpation des Pankreas (Leipzig, 1893); v. Noorden, Die Zuckerkrankheit (Berlin, 1901), which contains a very complete index of the literature. In regard to diabetes see also Cl. Bernard, Leçons sur le diabète (Paris), Seegen, Die Zuckerbildung im Thierkörper (Berlin, 1890), and Pflüger, Des Glykogen, 2. Aufl., 1905, and especially v. Noorden's Hanb. d. Pathol. des Stoffwechsels, 2. Aufl., 1907, Bd. 2, Chapter I.

[4] See Falta "Ueber den Eiweissumsatz beim Diabetes mellitus." Berl. klin. Wochenschr., 1908, and Zeitschr. f. klin. Med., **66**; Gigon, Deutsch. Arch. f. klin. Med., **97**.

the liver loses weight more than the other parts of the body. PFLÜGER concludes from this that the liver in diabetes works actively, and is the most important seat of production of diabetic sugar.

PFLÜGER has found that in frogs the total extirpation of the duodenum causes a strong and continuous glycosuria and based upon his investigations and those of other investigators, he believes that a certain relation exists between the duodenum and pancreas diabetes. The question as to the occurrence of a *duodenal diabetes* has been the subject of numerous investigations but the works of EHRMANN, MINKOWSKI and ROSENBERG [1] show that such a view is untenable.

There does not seem to be any doubt as to the existence of a certain relationship between the pancreas to the adrenals and adrenalin glycosuria. The glycosuric action of adrenalin could be prevented by ZUELZER by the injection of pancreas extracts, and this statement is confirmed by FRUGONI by experiments with pancreatic juice or pancreatic extracts. v. FÜRTH and SCHWARZ [2] have confirmed the correctness of ZUELZER'S statement but dispute the fact that we are here dealing with an antagonistic hormone action as they have obtained similar results also with other bodies, for example with turpentine.

Very stimulating views on the relationship of pancreas diabetes to the adrenals and the thyroids have been given by FALTA, EPPINGER and RUDINGER.[3] According to these investigators a reciprocal retardation exists between the pancreas and thyroid as between the pancreas and the adrenals while a mutual accelerating action exists between the thyroids and the adrenals. In depancreatized dogs the retarding action of the pancreas upon the thyroids is removed, and in this way we explain the strong increase in the protein, fat (MOHR) and salt-metabolism (FALTA and WHITNEY [4]) observed in pancreas diabetes. By the removal of the retarding action of the pancreas upon the adrenals, the mobilisation of the carbohydrates by means of the adrenalin is increased, and herein, as well as the diminished sugar utilization, lies the reason for the strong elimination of sugar. The relations between the above three glands is still further described by the above-mentioned authors, but we cannot enter more into detail in regard to the interesting question, which requires further study.

The conditions in pancreas diabetes are certainly very complicated, and the reasons for this are still very uncertain. Most investigators are of

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[1] Rosenberg, Bioch. Zeitschr., 18, which contains the literature.

[2] Frugoni, Berl. klin. Wochenschr., 45, 1908; v. Fürth and Schwarz, Bioch. Zeitschr., 31.

[3] Eppinger, Falta and Rudinger, Zeitschr. f. klin. Med., 66, which also contains the literature on adrenalin diabetes.

[4] Mohr, Zeitschr. f. exp. Path. u. Therap., 4; Falta and Whitney, Hofmeister's Beiträge, 11.

the opinion that we are here dealing with the abolition of one or more bodies which are considered as products of the internal secretion of the glands (*hormones* according to STARLING) and which in an unknown manner regulate the sugar destruction or carbohydrate metabolism.

The assumption of an internal secretion is based on the investigations of MINKOWSKI, HÉDON, LANCERAUX, THIROLOIX, and others[1] upon the action of the subcutaneous transplantation of the gland. According to these investigations a subcutaneously transplanted piece of the gland can completely perform the functions of the pancreas as to the sugar exchange and the sugar elimination, because on the removal of the intra-abdominal piece of gland, the animal in this case does not become diabetic, but if the subcutaneously embedded piece of pancreas is subsequently removed, an active elimination of sugar appears immediately. As this occurs also on completely cutting off the nerve supply, it is explained by the assumption of a formation of a special product in the gland, which passes into the blood; on the other hand ZUELZER, DOHM and MARXER[2] have made preparations from the pancreas which, in dogs as well as in man, cause a diminution in the elimination of sugar (and acetone bodies) in diabetes and an improvement in the general condition.

This internal secretion of the pancreas has in recent times been supposed to be connected with the so-called islands of LANGERHANS; but no positive results have been obtained in this connection. Nor are we acquainted with the kind of active substance here formed.

The glycolytic property of the blood as shown by LÉPINE was considered for a time to be due to a glycolytic enzyme formed in the pancreas, and pancreas diabetes used to be explained by the fact that the action of this enzyme was removed when the gland was extirpated. This glycolysis is not sufficient, even if it is derived from the pancreas, to explain the transformation of the large quantity of sugar in the body, and for the destruction of sugar we are also obliged to accept a glycolysis in the organs and tissues. Opinions in regard to this glycolysis differ in certain points. According to one view (SPITZER and others) special oxidases are active in the glycolysis, while another (STOKLASA[3]) considers the glycolysis as analogous to alcoholic fermentation, where we have processes brought on by special tissue zymases, in which lactic

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[1] See Minkowski, Arch. f. exp. Path. u. Pharm., **31**; Hédon, Diabète pancréatique, Travaux de Physiologie (Laboratoire de Montpellier, 1898), and the works on diabetes.

[2] Deutsch. med. Wochenschr., 1908.

[3] Hofmeister's Beiträge, **3**, Centralbl. f. Physiol., **16**, **17**, **18**; Ber. d. d. chem. Gesellsch., **38**; also with Czerny, *ibid.*, **36**; with Jelinek, Šimaček and Vitek, Pflüger's Arch., **101**.

acid is an intermediary step. Many [1] objections have been advanced against the view of STOKLASA that in animal as well as in plant tissues, in anærobic respiration, an alcoholic fermentation may occur as this observed action of the tissues could only be brought about by the presence of micro-organisms.

That lactic acid can be an intermediary step in the destruction of sugar in the animal body cannot be denied. On the contrary it follows from several circumstances which will be mentioned in Chapter X. (muscle) on the origin of lactic acid that such a condition exists and the following observations of A. R. MANDEL and LUSK [2] on the relation of lactic acid to diabetes indicate the same. These experimenters showed after phosphorus poisoning in dogs, that the blood and urine contained abundance of lactic acid, and on producing phlorhizin-diabetes it disappeared from these fluids, and also that phosphorus poisoning does not cause a lactic acid formation in dogs with phlorhizin-diabetes. Although it is difficult to give a satisfactory interpretation of these observations, it is still very probable that in the elimination of the sugar in phlorhizin-diabetes a mother-substance of the lactic acid is lost.

We do not agree as to the ways and means which bring about the so-called glycolysis, and another disputed question is whether the glycolysis can be produced by one organ or only by the combined action of several organs. COHNHEIM [3] found that a cell-free fluid can be obtained from a mixture of pancreas and muscle, which destroys glucose, while the pancreas alone does not have this action, and the muscle only to a slight extent. The pancreas does not contain, according to COHNHEIM, a glycolytic enzyme, but a substance resistant to boiling temperatures, which is soluble in water and alcohol, and which, like an amboceptor, activates a glycolytic proenzyme which exists in the muscle fluid, but which is inactive alone and which retards glycolysis when it exists in excess.

The statements of COHNHEIM have been disputed, and recently LEVENE and MEYER [4] have shown that we are not here dealing with a disappearance of glucose by glycolysis, but more likely with a disappearance

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[1] See the works of O. Cohnheim, Zeitschr. f. physiol. Chem., **39**, **42**, **43**; Batelli, Compt, rend., **127**; Portier, Compt. rend. soc. biol., **57**; Harden and Maclean, Journ. of Physiol., **42** and **43**.

[2] Amer. Journ. of Physiol., **16**.

[3] Cohnheim, Zeitschr. f. physiol. Chem., **39**, **42**, **43**, and **47**.

[4] Stocklasa and collaborators, Centralbl. f. Physiol., **17**, and Ber. d. d. chem. Gesellsch., **36** and **38**; Feinschmidt, Hofmeister's Beiträge, **4**; Hirsch, *ibid.*; Claus and Embden, *ibid.*, **6**; Arnheim and Rosenbaum, Zeitschr. f. physiol. Chem., **40**; Braunstein, Zeitschr. f. klin. Med., **51**; Levene and Meyer, Journ. of biol. Chem., **9**.

due to synthesis, where a disaccharide is formed. According to J. DE MEYER [1] neither the pancreas nor the tissues as a whole contain any glycolytic enzymes. According to him only the blood has a glycolytic action, and this action is supported by a body acting as an amboceptor and produced in the pancreas. Our knowledge as to the existence of the glycolysis and the mode of action of the pancreas in the metabolism of sugar in the animal body is very meager and incomplete.

Where does the sugar eliminated in diabetes originate? Does it depend entirely upon the carbohydrates of the food or the store of carbohydrates in the body, or has the body the power of producing sugar from other material? To LÜTHJE belongs the credit for positively deciding this question. He has made experiments on dogs with pancreas diabetes, in which on a protein diet free from carbohydrates so much sugar was eliminated that it could not possibly be accounted for by the store of glycogen or other carbohydrate-containing substances in the body. Similar experiments were also performed later by PFLÜGER,[2] with the results that the power of the animal body to produce sugar from non-carbohydrate material is now definitely proved.

Is this sugar produced from protein or fat, or from both? This question so far has not been answered, and it is the subject of continuous dispute. It is not possible to enter into an exhaustive and detailed discussion of the question in a text-book, and we will only mention, briefly, certain of the most important observations and historical points.

The largest amount of sugar which we can obtain theoretically from protein is 8 grams of sugar from 1 gram of protein nitrogen, if we admit that all the carbon of the protein, with the exception of that necessary to form ammonium carbonate, is used for the formation of sugar. These results are still somewhat too high for the average carbon and nitrogen content of the proteins and the values D:N = 6.6 is probably more correct.[3] The actual relation between glucose and nitrogen in the urine, i.e., the quotient D: N, has been repeatedly determined in various forms of diabetes, and in depancreatized dogs it is generally 2.8 and in starving dogs or dogs fed with protein and poisoned with phlorhizin it is equal to 3.65 (LUSK). It may undergo considerable variation, and in certain cases it may indeed be lower than 1 as well as higher than 8, and high results have been repeatedly obtained in cases of human diabetes. From these quotients conclusions have been drawn as to the amount of sugar

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[1] Cited from Centralbl. f. Physiol., **20** and **23**. See also Lépine, État actuel de la question de la Glycolyse, La semaine médicale, 1911.

[2] Lüthje, Deutsch. Arch. f. klin. Med., **79**, and Pflüger's Arch., **106**; Pflüger, Pflüger's Arch., **108**.

[3] See Falta, Zeitschr. f. klin. Med., **65**; see also Gigon, Deutsch. Arch. f. klin. Med., **97**.

formed, as well as the origin of the sugar, but according to the views of HAMMARSTEN such conclusions are mostly very uncertain. The sugar eliminated by the urine represents the difference between the total sugar production of the body and the quantity of sugar burned or utilized. Only under the supposition that the body cannot burn or utilize any sugar, is the sugar of the urine a measure of the quantity produced, and this seems to be the case in phlorhizin diabetes; but it is difficult to decide how these suppositions apply to the different forms of diabetes. Still several observations seem to show that in the different forms of diabetes variable amounts of the sugar are burned, and only in special cases can we draw approximately accurate conclusions.

The property of protein of increasing the elimination of sugar is considered as an important proof of the formation of sugar from protein. In this regard those experiments are of special interest in which the diabetic animal is allowed to starve until the urine is poor in sugar or indeed free from sugar, and then on feeding with protein, an abundant elimination of sugar is produced. If we do not accept the view in this case that the protein, but rather the fat, was the material from which the sugar was produced, still we must admit either of a sugar-sparing action due to protein or of a strong sugar formation from fat, incited by the protein.

A sparing in the sense that the protein is oxidized instead of the sugar, and in this manner protects it, is naturally possible only under the supposition that the body can burn at least a part of the sugar, otherwise there would be nothing to spare and nothing to protect from burning. The assumption of such an indirect action of proteins is difficult to reconcile with the common view of the inability of the body to burn sugar in diabetes. LÜTHJE[1] has communicated one experiment among others, in which a dog with pancreas diabetes, whose weight before starvation was 18 kilos, with nineteen days' starvation eliminated an average of 10.4 grams sugar for the last six days of starvation. By exclusive protein feeding the quantity of sugar per day could be raised to a maximum of 123.6 grams, and as average it was 97.5 grams for the ten protein days. The protein, therefore, had protected daily an average of 87 grams sugar from burning, which is hardly possible; and if in the diabetic animal we admit of this considerable power of burning sugar, the quotient D:N becomes valueless as a measure of the quantity of sugar formed.

If, on the contrary, we admit of an indirect action of proteins in that they incite a sugar formation from fat, perhaps by a certain very important increase in the activity of the liver, we are opposed by the great difficulty that, according to known laws of metabolism, the pro-

[1] Deutsch. Arch. f. klin. Med., 79.

teins do not raise the fat metabolism, but rather diminish it. The protein displaces a corresponding quantity of fat from the metabolism, and if the fat were the only source of sugar then in this case we would expect a diminished elimination of sugar instead of an increased one. Nevertheless the above action of protein upon sugar elimination is much more easily explained by the assumption of a sugar formation from protein than from fat.

The action of monamino-acids upon the carbohydrate metabolism has also given important ground for the assumption of a sugar formation from protein. That a deamidation occurs in the animal body was shown by the earlier observations of BAUMANN and BLENDERMANN. Further proofs of this were furnished by the investigations of NEUBERG and LANGSTEIN, where in feeding experiments with alanine they found abundance of lactic acid in the urine, and P. MAYER [1] observed glyceric acid in the urine after the subcutaneous injection of diaminopropionic acid. As from amino-acids by deamidation ketone acids or oxyacids may be formed (see Chapter XIV) it would be of interest to test the action of amino-acids upon the carbohydrate metabolism. Several investigations have been carried on with this in view, such as those of LANGSTEIN and NEUBERG, R. COHN and F. KRAUS, which have shown a very probable formation of carbohydrate under the influence of amino-acids; but the investigations of EMBDEN and SALOMON, and of EMBDEN and ALMAGIA have positively shown, in a dog without a pancreas, that the amino-acids can bring about a re-formation of carbohydrate. LUSK alone and with RINGER [2] have shown the same for several amino-acids by experiments on dogs poisoned with phlorhizin. According to the experiments and calculations of the two last mentioned investigators glycocoll and alanine can be completely transformed into glucose. Of the four carbon atoms of aspartic acid and of the five carbon atoms of glutamic acid three appear as glucose.

The investigations of WEINLAND [3] tend to prove a sugar formation from protein. He studied the formation of sugar in the chrysalis pulp of the Calliphora and showed that the sugar formed thereby did not originate from the fat, but that the protein was the only material from

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[1] Baumann, Zeitschr. f. physiol. Chem., **4**; Blendermann, *ibid.*, **6**; Neuberg and Langstein, Arch. f. (Anat. u.) Physiol., 1903, Suppl.; Mayer, Zeitschr. f. physiol. Chem., **42**.

[2] Langstein and Neuberg, l. c.; Cohn, Zeitschr. f. physiol. Chem., **28**; F. Kraus, Berl. klin. Wochenschr., 1904; Embden and Salomon, Hofmeister's Beiträge, **5** and **6**, and with Almagia, *ibid.*, **7**; Lusk, Amer. Journ. of Physiol., **22**; Ringer and Lusk, Zeitschr. f. physiol. Chem., **66**.

[3] Zeitschr. f. Biol., **49** (N. F., 31); with Krummacher, *ibid.*, **52**.

which the sugar was formed. The formation of sugar from protein is now generally considered as positively proved.

DAKIN [1] has found with experiments with phlorhizinized dogs that serine, cysteine, proline, ornithine and arginine yield abundant sugar in glycosuric animals. Valine, leucine, isoleucine, lysine, histidine, phenylalanine and tryptophane gave relatively little sugar or none at all. The amino-acids with straight chains (with the exception of lysine) give sugar while those with branched chains do not. Proline is the only cyclic amino-acid, which yields abundance of sugar. Arginine is the only one with more than five carbon atoms which yields sugar and the sugar comes in this case from the ornithine components.

If we assume a formation of sugar from fat, we must differentiate between the two components of neutral fats, that is, between the glycerin and the fatty acids. A formation of sugar from glycerin can be considered as proved by the investigations of CREMER, and especially those of LÜTHJE [2] and in the following we will discuss only the formation of sugar from the fatty acids.

The formation of sugar from fat seems to occur in the plant kingdom, and as the chemical processes in the animal and plant life are in principle the same, it makes the possibility of a sugar formation from fat very probable. Such an origin of sugar in the animal body is accepted by many investigators, especially by PFLÜGER and several French observers, among whom we must specially mention CHAUVEAU and KAUFMANN.[3]

When food as free from carbohydrate as possible is taken, the quotient D:N is high, i.e., higher than 8, as well as when the quantity of sugar is so large that it cannot be accounted for by the calculated protein (and carbohydrate) metabolism, then if the observations are otherwise free from error we can admit of a formation of sugar from fat. Several such cases of diabetes in man have been published (RUMPF, ROSENQVIST, MOHR, v. NOORDEN, ALLARD, FALTA and co-workers and others), and also in animals (HARTOGH and SCHUMM [4]). Although these researches are not fully conclusive, still certain of them indicate a probable formation of sugar from fat. We also have several conditions which

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[1] Journ. of biol. Chem., **14**, 321.

[2] Cremer, Sitzungsber. d. Ges. f. Morph. u. Physiol. München, 1902; Lüthje, Deutsch. Arch. f. klin. Med., **80**.

[3] Kaufmann, Arch. f. Physiol. (5), 8, where Chauveau's work is cited.

[4] Rumpf, Berl. klin. Wochenschr., 1899; Rosenqvist, *ibid.*; Mohr, *ibid.*, **1901**; v. Noorden, Die Zuckerkrankheit, 3. Aufl. Berlin, 1901; Allard, Arch. f. exp. Path. u. Pharm., **57**; Falta and co-workers, Zeitschr. f. klin. Med., **66**; Hartogh and Schumm, Arch. f. Path. u. Pharm., **45**. See also the works of O. Loewi, *ibid.*, **47**, and Lusk, Zeitschr. f. Biologie, **42**.

indicate the same, namely, that in phlorhizin diabetes after the disappearance of the liver-glycogen the fat which migrates to the liver serves as material for the formation of sugar (PFLÜGER). These observations make the formation of sugar from fat highly probable and the same is true for the observations of JUNKERSDORF.[1] He found that in an animal made glycogen free, by starvation and with phlorhizin poisoning, that toward death, the nitrogen as well as the sugar elimination increased but that the D:N ratio was higher than with the sugar formation from protein alone. His calculations are not free from exception.

On the other hand there are many observations on animals and also clinical observations which oppose the theory of the formation of sugar from fat in diabetes. LUSK found in a dog with phlorhizin diabetes that the quotient D:N=3.65:1 was not changed on feeding fat, and he has published further results of experiments[2] which show that active muscular work, which strongly increases the fat decomposition, does not change the quotient in dogs with phlorhizin diabetes. It is difficult to draw positive conclusions from these experiments, still LUSK seems to deny the formation of sugar from fat.

Attempts have been made to solve the question as to the material from which sugar is formed by the determination of the respiratory quotient and comparing this with the quotient D:N. The calculations in this direction have not led to positive results.[3] As the quotient D:N is not an accurate measure of the quantity of sugar formed, and as we, as yet, do not know the quantity of oxygen necessary to form sugar from protein, HAMMARSTEN believes that it is just as impossible to conclude from the respiratory quotient that sugar is formed from the fats as from the proteins.

We have no complete proofs for the formation of sugar from fat, still we can indicate the probable proofs therefor. There is really no objection from a theoretical standpoint to the assumption that the body has the power of producing sugar from protein as well as from fat, and such a power does not seem improbable.

As a formation of sugar from protein is now generally considered as proved, it follows that the protein can yield material for the formation of glycogen and that it is a true glycogen-former. PFLÜGER and JUNKERSDORF[4] have given direct proof for this. They fed a dog, which had previously been made glycogen-free by starvation and phlorhizin injec-

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[1] Pflüger's Arch., **137**.

[2] Amer. Journ. of Physiol., **22**.

[3] Magnus-Levy, Zeitschr. f. klin. Med., **56**; Pflüger's Arch., **108**; Mohr, Zeitschr. f. exp. Path. u. Therap., **4**.

[4] Pflüger's Arch., **131**.

tions, with abundance of codfish and then found so much glycogen (6.46 per cent in the liver and 1 per cent in the muscle) that a re-formation of glycogen must have undoubtedly occurred. By special control experiments with fat feeding they also showed that the glycogen did not originate from the fat but must unquestionably have come from the protein. Carbohydrates and proteins are without question true glycogen-formers, while the question in regard to fats is still open.

The Bile and Its Formation.

By the establishment of a biliary fistula, an operation which was first performed by SCHWANN in 1844 and which has been improved lately by DASTRE and PAWLOW,[1] it is possible to study the secretion of the bile. This secretion is continuous, but with varying intensity. It takes place under a very low pressure; therefore an apparently unimportant hindrance in the outflow of the bile, namely, a stoppage of mucus in the exit, or the secretion of large quantities of viscous bile, may cause stagnation and absorption of the bile by means of the lymphatic vessels (absorption icterus).

The quantity of bile secreted in the twenty-four hours in dogs can be exactly determined. The quantity secreted by different animals varies, and the limits are 2.9–36.4 grams of bile per kilo of weight in the twenty-four hours.[2]

The reports as to the extent of bile secretion in man are few and not to be depended on. NOËL-PAYTON, MAYO-ROBSON, HAMMARSTEN, PFAFF and BALCH, and BRAND[3] found a variation between 514 and 1083 cc. per twenty-four hours. Such determinations are of doubtful value, because in most cases it follows from the composition of the collected bile that the fluid is not the result of a secretion of normal liver bile.

The quantity of bile secreted is, however, as shown by STADELMANN,[4] subject to such great variation, even under physiological conditions, that the study of those circumstances which influence the secretion is very difficult and uncertain. The contradictory statements by different investigators may probably be explained by this fact.

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[1] Schwann, Arch. f. (Anat. u.) Physiol., 1844; Dastre, Arch. de Physiol. (5) 2; Pawlow, Ergebnisse der Physiol., 1, Abt. 1.

[2] In regard to the quantity of bile secreted in animals see Heidenhain, Die Gallenabsonderung, in Hermann's Handbuch der Physiol., 5, and Stadelmann, Der Icterus und seine verschiedenen Formen (Stuttgart, 1891).

[3] Noël-Payton, Rep. Lab. Roy. Coll. Edinburgh, 8; Mayo-Robson, Proc. Roy. Soc., 47; Hammarsten, Nova Act. Reg. Soc. Scient. Upsala (3), 16; Pfaff and Balch, Journ. of Exp. Med., 1897; Brand, Pflüger's Arch., 90.

[4] Stadelmann, Der Icterus, etc., Stuttgart, 1891.

In starvation the secretion diminishes. According to LUKJANOW and ALBERTONI,[1] under these conditions the absolute quantity of solids decreases, while the relative quantity increases. After partaking of food the secretion increases again. The findings are very contradictory in regard to the time necessary, after partaking of food, before the secretion reaches its maximum. After a careful examination and compilation of all the existing reports, HEIDENHAIN[2] has come to the conclusion that in dogs the curve of rapidity of secretion shows two maxima, the first at the third to fifth hour and the second at the thirteenth to fifteenth hour after partaking of food. According to BARBÉRA the time when the maximum occurs is dependent upon the kind of food. With carbohydrate food it is two to three hours, after protein food three to four hours, and with fat diet it is five to seven hours, after feeding. According to LOEB[3] the maximum occurs in dogs one to two hours after feeding with meat, casein or gliadin.

According to earlier observations, the proteins of all the various foods cause the greatest secretion of bile, while the carbohydrates diminish the secretion, or at least excite it much less than the proteins. This coincides with the recent observations of BARBÉRA. The authorities by no means agree as to the action of the fats. While many older investigators have not observed any increase, but rather the reverse in the secretion of bile after feeding with fats, the researches of BARBÉRA show an undoubted increase in the secretion of bile on fat feeding, greater even than after carbohydrate feeding. According to ROSENBERG olive-oil is a strong cholagogue, a statement which, according to other investigators—MANDELSTAMM, DOYON and DUFOURT[4]—has not been proved.

As BARBÉRA has shown, a close relation exists between the bile secretion and the quantity of urea formed, as an increase in the first goes hand in hand with an increase of the latter. The bile is, therefore, according to him, a product of disassimilation, whose quantity rises and falls with the degree of activity of the liver.

The question whether there exists special medicinal bodies, so-called *cholagogues*, which have a specific excitant action on the secretion of

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[1] Lukjanow, Zeitschr. f. physiol. Chem., **16**; Albertoni, Recherches sur la sécrétion biliaire, Turin, 1893.

[2] Hermann's Handb., **5**, and Stadelmann, Der Icterus, etc.

[3] Barbéra, Centralbl. f. Physiol., **12** and **16**; A. Loeb, Zeitschr. f. Biol., **55**.

[4] Barbéra, Bull. della scienz. med. di Bologna (7), **5**, Maly's Jahresber., **24**, and Centralbl. f. Physiol., **12** and **16**; Rosenberg, Pflüger's Arch., **46**; Mandelstamm, Ueber den Einfluss einiger Arzneimittel auf Sekretion und Zusammensetzung der Galle (Dissert. Dorpat, 1890); Doyon and Dufourt, Arch. de Physiol. (5), **9**. In regard to the action of various foods on the secretion of bile see also Heidenhain, l. c.; Stadelmann, Der Icterus; and Barbéra, l. c.

bile, has been answered in very different ways. Many, especially the older investigators, have observed an increase in the bile secretion after the use of certain therapeutic agents, such as calomel, rhubarb, jalap, turpentine, olive-oil, etc.; while others, especially the more recent investigators, have arrived at quite opposite results. From all appearances this contradiction is due to the great irregularity of the normal secretion, which might readily cause mistakes in tests with therapeutic agents.

SCHIFF'S view, that the bile absorbed from the intestinal canal increases the secretion of bile and hence acts as a cholagogue, seems to be a positively proved fact by the investigations of several experimenters.[1] Sodium salicylate is also perhaps a cholagogue (STADELMANN, DOYON and DUFOURT, WINOGRADOW) and according to PETROWA [2] in dogs sodium benzoate, thymole, phenol, menthol and all such bodies which are conjugated to ethereal sulphuric acid in the animal body, increase the secretion of bile.

Acids, and especially, under normal conditions, hydrochloric acid, seem to be physiological excitants for bile secretion. According to FALLOISE and FLEIG the acids act upon the duodenum and the upper part of the jejunum, and the action is brought about by a secretin formation similar to the action of acids upon the secretion of pancreatic juice (see Chapter VIII). According to FALLOISE [3] chloral hydrate introduced into the duodenum causes a secretion of bile in an analogous manner, by the aid of a special *chloral secretin*.

The bile is a mixture of the secretion of the liver-cells and the so-called mucus which is secreted by the glands of the biliary passages and by the mucous membrane of the gall-bladder. The secretion of the liver, which is generally poorer in solids than the bile from the gall-bladder, is thin and clear, while the bile collected in the gall-bladder is more ropy and viscous on account of the absorption of water and the admixture of "mucus," and cloudy because of the presence of cells, pigments, and the like. The specific gravity of the bile from the gall-bladder varies considerably, being in man between 1.010 and 1.040. Its reaction is alkaline to litmus. The color changes in different animals: golden-yellow, yellowish-brown, olive-brown, brownish-green, grass-green or bluish-green. Bile obtained from an executed person immediately after death is golden-yellow or yellow with a shade of brown. Still cases

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[1] Schiff, Pflüger's Arch., **3**. See Stadelmann, Der Icterus, and the dissertations of his pupils, especially Winteler, "Experimentelle Beiträge zur Frage des Kreislaufes der Galle" (Inaug.-Diss. Dorpat, 1892), and Gärtner, "Experimentelle Beiträge zur Physiol. und Path. der Gallensekretion" (Inaug.-Dis. Jurjew, 1893); also Stadelmann, "Ueber den Kreislauf der Galle," Zeitschr. f. Biologie, **34**.

[2] Zeitschr. f. physiol. Chem., **74** (literature). See also footnote 4, page 415.

[3] Falloise, Bull. Acad. Roy. de Belg., 1903; Fleig, *ibid.*, 1903.

occur in which fresh human bile from the gall-bladder has a green color. The ordinary post-mortem bile has a variable color. The bile of certain animals has a peculiar odor; for example, ox-bile has an odor of musk, especially on warming. The taste of bile is also different in different animals. Human as well as ox-bile has a bitter taste, with a sweetish after-taste. The bile of the pig and rabbit has an intensely persistent bitter taste. On heating bile to boiling it does not coagulate. It contains (in the ox) only traces of true mucin, and its ropy properties depend, it seems, chiefly on the presence of a nucleoalbumin similar to mucin (PAIJKULL). The bile from the animals investigated by HAMMARSTEN showed a similar behavior. HAMMARSTEN[1] has, on the contrary, found a true mucin in human bile. To all appearances this mucin originates from the billiary passages, as he found it in the bile flowing from the hepatic duct, and also because the mucous membrane of the gall-bladder, according to WAHLGREN,[2] does not in man secrete any mucin, but a mucin-like nucleoalbumin.

The specific constituents of the bile are *bile-acids* combined with alkalies, *bile-pigments*, and, besides small quantities of *lecithin* and *phosphatides*, *cholesterin*, *soaps*, *neutral fats*, *urea*, *ethereal sulphuric acid*, traces of *conjugated glucuronic acids*, *enzymes* and *mineral substances*, chiefly chlorides, besides phosphates of calcium, magnesium, and iron. Traces of copper also occur.

Bile-salts. The bile-acids, which thus far have best been studied, may be divided into two groups, the *glycocholic* and *taurocholic acid* groups. As found by HAMMARSTEN[3] a third group of bile-acids occurs in the shark, which are rich in sulphur, and like the ethereal sulphuric acids they split off sulphuric acid on boiling with hydrochloric acid. All glycocholic acids contain nitrogen, but are free from sulphur and can be split, with the addition of water, into glycocoll (amino-acetic acid) and a nitrogen-free acid, a cholic acid. All taurocholic acids contain nitrogen and sulphur and are split, with the addition of water, into taurine and a cholic acid. The reason for the existence of different glycocholic and taurocholic acids depends on the fact that there are several cholic acids.

The conjugated bile-acid found in the shark, and called *scymnol-sulphuric acid* by HAMMARSTEN, yields as cleavage products sulphuric acid and a non-nitrogenous substance, *scymnol* ($C_{27}H_{46}O_5$), which gives the characteristic color reactions of cholic acid.

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[1] Paijkull, Zeitschr. f. physiol. Chem., **12**; Hammarsten, l. c., Nova Act. (3), **16**, and Ergebnisse der Physiol., Bd. **4**.

[2] Maly's Jahresber., **22**.

[3] Hammarsten, Zeitschr. f. physiol. Chem., **24**.

The different bile-acids occur in the bile as alkali salts, generally the sodium compounds, even in sea-fishes, although this is contrary to the earlier observations (ZANETTI [1]). In the bile of certain animals we find almost solely glycocholic acid, in others only taurocholic acid, and in still others a mixture of both (see below).

All alkali salts of the biliary acids are soluble in water and alcohol, but insoluble in ether. Their solution in alcohol is therefore precipitated by ether, and this precipitate, with proper care in manipulation, gives, for nearly all kinds of bile thus far investigated, rosettes or balls of fine needles, or four- to six-sided prisms (PLATTNER'S crystallized bile). Fresh human bile also crystallizes readily. The bile-acids and their salts are optically active and dextrorotatory. The salts of the different bile-acids act somewhat differently toward neutral salts. The alkali salts of the ordinary and best-studied bile-acids from man, ox, and dog are, according to TENGSTRÖM,[2] precipitated by ammonium and magnesium sulphates, and also, in pure form, by sodium nitrate and sodium chloride (added to saturation). Potassium and sodium sulphates do not precipitate them. The alkali salts cannot be directly precipitated from the bile by NaCl, on account of the presence of bodies retarding precipitation, among which we find oil-soaps.

The bile-acids are dissolved by concentrated sulphuric acid at the ordinary temperature, forming a reddish-yellow liquid which has a beautiful green fluorescence. According to PREGL an oxidation with a reduction of the sulphuric acid into sulphur dioxide takes place. The fluorescent substance has been called dehydrocholan (see below) by PREGL.[3] On carefully warming with concentrated sulphuric acid and a little cane-sugar, the bile-acids give a beautiful cherry-red or reddish-violet liquid. PETTENKOFER'S reaction for bile-acids is based on this behavior.

PETTENKOFER'S *test for bile-acids* is performed as follows: A small quantity of bile in substance is dissolved in a small porcelain dish in concentrated sulphuric acid and warmed, or some of the liquid containing the bile-acids is mixed with concentrated sulphuric acid, taking special care in both cases that the temperature does not rise higher than 60–70° C. Then a 10-per-cent solution of cane-sugar is added, drop by drop, continually stirring with a glass rod. The presence of bile is indicated by the production of a beautiful red liquid, whose color does not disappear at the ordinary temperature, but becomes more bluish-violet in the course of a day. This red liquid shows a spectrum with two absorption-bands, the one at *F* and the other between *D* and *E*, near *E*.

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[1] See Chem. Centralbl., 1903, **1**, 180.
[2] Zeitschr. f. physiol. Chem., **41**.
[3] Zeitschr. f. physiol. Chem., **45**.

This extremely delicate test fails, however, when the solution is heated too high, or if an improper quantity—generally too much—of the sugar is added. In the last-mentioned case the sugar easily carbonizes and the test becomes brown or dark brown. The reaction fails if the sulphuric acid contains sulphurous acid or the lower oxides of nitrogen. Many other substances, such as proteins, oleic acid, amyl alcohol, and morphine, give a similar reaction, and therefore in doubtful cases the spectroscopic examination of the red solution must not be forgotten.

PETTENKOFER'S test for the bile-acids depends essentially on the fact that furfurol is formed from the sugar by the sulphuric acid (MYLIUS). According to MYLIUS and V. UDRANSZKY [1] a 1 p. m. solution of furfurol should be used. Dissolve the bile, which must first be decolorized by animal charcoal, in alcohol. To each cubic centimeter of alcoholic solution of bile in a test-tube add 1 drop of the furfurol solution and 1 cc. concentrated sulphuric acid, and cool when necessary, so that the test does not become too warm. This reaction, when performed as described, will detect $\frac{1}{10}$ to $\frac{1}{50}$ milligram cholic acid (V. UDRANSZKY). Other modifications of PETTENKOFER'S test have been proposed.

The reaction with furfurol is not identical with that obtained with cane-sugar, according to VILLE and DERRIEN, and the absorption-bands do not occur in the same place in the two cases. The reaction with cane-sugar does not depend, according to these investigators, upon a furfurol formation from the sugar. The acid hydrolyzes the sugar, and from the fructose produced, 4-methyl-2-oxyfurfurol is formed by the further action of the acid, and this gives the color reaction with the cholic acid. Instead of furfurol other aldehydes such as vanillin and anisaldehyde can be used according to VILLE and DERRIEN.[2]

Glycocholic Acid. The constitution of the glycocholic acid occurring in human and ox-bile, and which has been most studied, is represented by the formula $C_{26}H_{43}NO_6$. Glycocholic acid is absent, or nearly so, in the bile of carnivora. On boiling with acids or alkalies this acid, which is analogous to hippuric acid, is converted into cholic acid and glycocoll.

By the action of hydrazine hydrate upon the ethyl ester of cholic acid BONDI and MÜLLER [3] prepared first cholic-acid hydrazide, and then, by the action of nitrous acid upon this, they obtained the cholic-acid azide, $C_{23}H_{39}O_3CO.N_3$, and finally from this last in alkaline solution with glyco-

[1] Mylius, Zeitschr. f. physiol. Chem., 11; v. Udranszky, *ibid.*, 12.

[2] Ville and Derrien, Chem. Centralbl. 1909, 2, 1699 and Compt. rend. soc., biol. 64 and 66.

[3] Zeitschr. f. physiol. Chem., 47.

coll they synthetically prepared the alkali salt of glycocholic acid, at the same time splitting off nitrogen.

Glycocholic acid crystallizes in fine, colorless needles or prisms. It is soluble with difficulty in water (in about 300 parts cold and 120 parts boiling water), and is easily precipitated from its alkali-salt solution by the addition of dilute mineral acids. According to BONDI [1] glycocholic acid is a rather strong acid, about as acid as lactic but much stronger than acetic acid. This last-mentioned acid precipitates glycocholic acid from the solution of its alkali salts in water. It is readily soluble in strong alcohol, but with great difficulty in ether. The solutions have a bitter but at the same time sweetish taste. The acid melts between 132–152°, depending upon the method of preparation. According to LETSCHE, the acid containing water of crystallization (1½ mol.) deflagrates on heating rapidly at 126°, and at 130° an active frothing is observed. The acid free from water of crystallization deflagrates at 130–132°, and decomposes at 154–155° C. with frothing. The salts of the alkalies and alkaline earths are soluble in alcohol and water.

The solution of the alkali salt in water can be salted out by NaCl, but not by KCl. The salts of the heavy metals are mostly insoluble or soluble with difficulty in water. The solution of the alkali salts in water is precipitated by sugar of lead, cupric and ferric salts, and silver nitrate.

On boiling with water glycocholic acid is probably transformed into its physical isomer paraglycocholic acid, according to LETSCHE,[2] and this crystallizes in long leaves which, when containing water of crystallization, show ready deflagration at 186° and decompose with frothing at 198° C. On solution in alcohol or dilute alkalies the paraglycocholic acid passes into the ordinary glycocholic acid.

Glycocholeic Acid is a second glycocholic acid, first isolated by WAHLGREN [3] from ox-bile, and has the formula $C_{26}H_{43}NO_5$ or $C_{27}H_{45}NO_5$. This acid, which on hydrolytic cleavage yields glycocoll and choleic acid, has also been detected in human bile and the bile of the musk-ox (HAMMARSTEN [4]).

Glycocholeic acid may, like glycocholic acid, crystallize in tufts of fine needles, but is often obtained as short thick prisms. It is much more insoluble in water, even on boiling, than glycocholic acid, and it melts at 175–176° C. The alkali salts are soluble in water, have a pure bitter taste, and are more readily precipitated by neutral salts (NaCl) than the glycocholates. The solution of the alkali salts is not only precipitated

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[1] Zeitschr. f. physiol. Chem., **53**.
[2] *Ibid.*, **60** and **73**.
[3] *Ibid.*, **86**.
[4] *Ibid.*, **43**.

by the salts of the heavy metals, but also by the salts of barium, calcium and magnesium.

The principle in the preparation of the pure glycocholic acids consists in treating a 2–3 per cent solution of bile free from mucus, when rich in glycocholic acid (so-called HÜFNER'S bile [1]), with ether, and then with 2 per cent hydrochloric acid. If the bile is not directly precipitable with hydrochloric acid (bile relatively poor in glycocholic acid), then precipitate the chief mass of the glycocholic acid with ferric chloride, or better with lead acetate, decompose the precipitate with soda and treat the 2 per cent solution as above stated with ether and hydrochloric acid. The crystalline and washed mass is boiled with water, and on cooling glycocholic acid crystallizes out, and then this is recrystallized from water or from alcohol by the addition of water. The residue that remains after boiling in water (paraglycocholic acid and glycocholeic acid) is converted into their barium salts, and after a complicated method (see WAHLGREN) the glycocholeic acid is obtained. The reader is referred to more exhaustive works for other methods of preparation.

Hyoglycocholic Acid, $C_{27}H_{43}NO_6$, is the crystalline glycocholic acid obtained from the bile of the pig. It is very insoluble in water. The alkali salts, whose solutions have an intensely bitter taste, without any sweetish after-taste, are precipitated by $CaCl_2$, $BaCl_2$, and $MgCl_2$, and may be salted out like a soap by Na_2SO_4 when added in sufficient quantity. According to Piettre it can be salted out entirely, free from sulphur, by caustic alkali which is not possible by other methods. By precipitation with NaCl in such quantity that the precipitate redissolves on warming, HAMMARSTEN [2] obtained the alkali salt, as macroscopic crystals, on cooling. Besides this acid there occurs in the bile of the pig still another glycocholic acid (JOLIN [3]).

The glycocholate in the bile of rodents is also precipitated by the above mentioned earthy salts, but cannot, like the corresponding salt in human or ox-bile, be directly precipitated on saturating with a neutral salt (Na_2SO_4). **Guano bile-acid** possibly belongs to the glycocholic-acid group, and is found in Peruvian guano, but has not been thoroughly studied.

Taurocholic Acid. This acid, which is found in the bile of man, carnivora, oxen, and a few other herbivora, such as sheep and goats, has the constitution $C_{26}H_{45}NSO_7$. On boiling with acids and alkalies it splits into cholic acid and taurine. Taurocholic acid has also been prepared synthetically by BONDI and MÜLLER, using the same method as they used for glycocholic acid.

Taurocholic acid can be readily obtained, by the method suggested by HAMMARSTEN,[4] as groups of fine needles or as beautiful prisms on slow crystallization. The crystals do not change in the air, but they decompose above 100°. They are soluble in alcohol but insoluble in

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[1] Hüfner, Journ. f. prakt. Chem. (N. F.), **10**, **19**, and **25**.

[2] Not published. M. Piettre, Recherches sur la bile, Laval, 1910.

[3] Zeitschr. f. physiol. Chem., **12** and **13**.

[4] *Ibid.*, **43**.

ether, benzene, and acetone. Taurocholic acid is very soluble in water, and the solution has a very sweet taste, with only a slight bitter taste. It can hold the difficultly soluble glycocholic acid in solution. This is the reason why a mixture of glycocholate with a sufficient quantity of taurocholate, which often occurs in ox-bile, is not precipitated by a dilute acid. Its salts are, as a rule, readily soluble in water, and the solutions of the alkali salts are not precipitated by copper sulphate, silver nitrate or lead acetate. Basic lead acetate gives, on the contrary, a precipitate which is soluble in boiling alcohol. The alkali salts are not only precipitated from their solution by the same neutral salts that precipitate glycocholic acid, but also by potassium chloride, and by sodium and potassium acetates.

Taurocholeic Acid is a second taurocholic acid, detected by HAMMARSTEN in dog-bile and isolated by GULLBRING [1] from ox-bile, and has the formula $C_{26}H_{45}NSO_6$ or $C_{27}H_{47}NSO_6$. Thus far it has been obtained only in the amorphous form. It is readily soluble in water, and has a disagreeably bitter taste. It is also readily soluble in alcohol, but insoluble in ether, acetone, chloroform, and benzene. The alkali salt, soluble in water, can be salted out by NaCl as a pasty mass. The solutions of the salts can be precipitated by ferric chloride. The cleavage products are taurine and choleic acid.

The taurocholic acids are most simply prepared from bile, free from glycocholic acid or poor therein, such as fish- or dog-bile, easiest from the latter. The aqueous solution of the mucus-free bile is almost completely precipitated by ferric chloride. The precipitate is worked for taurocholeic acid and the filtrate for taurocholic acid. The iron is first removed from the filtrate by Na_2CO_3, and then the faintly alkaline filtrate saturated with NaCl. The taurocholate separates out and after further purification is decomposed by alcohol containing hydrochloric acid. The taurocholic acid is precipitated from the alcoholic filtrate by ether and recrystallized from alcohol containing water by the addition of ether. The taurocholeic acid is obtained from the above iron precipitate by treating it with soda, and decomposing the alkali salt of the taurocholeic acid with alcohol, containing HCl, and precipitating the acid from the alcoholic solution with ether and repeating this precipitation from alcohol by ether.

Cheno-taurocholic Acid. This is the most essential acid of goose-bile and has the formula $C_{28}H_{49}NSO_6$. This acid, but little studied, is amorphous and soluble in water and alcohol.

The taurocholic acids differ from the glycocholic acids in being readily soluble in water. In the bile of the walrus, on the contrary, a relatively insoluble, readily crystallizable taurocholic acid occurs, which

[1] Hammarsten, Zeitschr. f. physiol. Chem., **43**; Gullbring, *ibid.*, **45**.

can be precipitated from the solution of the alkali salts by the addition of mineral acids, like glycocholic acid (HAMMARSTEN [1]).

As repeatedly mentioned above, the two bile-acids split on boiling with acids or alkalies into non-nitrogenous cholic acids and into glycocoll or taurine. Of the various cholic acids the following have been best studied.

Cholic Acid or Cholalic Acid. The ordinary cholic acid obtained as a decomposition product of human and ox-bile, which occurs, regularly in the contents of the intestine, and also in the urine in icterus, has, accordint to STRECKER and nearly all recent investigators, the constitution $C_{24}H_{40}O_5, = C_{20}H_{31}\begin{cases}CHOH\\(CH_2OH)_2\\COOH\end{cases}$. According to MYLIUS,[2] cholic acid is a monobasic alcohol-acid with one secondary and two primary alcohol groups. CURTIUS [3] has shown by preparing the cholamine, $C_{23}H_{39}O_3.NH_2$, from the above-mentioned (p. 419) cholic-acid azide, with cholic-acid urethane as an intermediary step, that the carboxyl group is not immediately connected with the CHOH group, but is combined with the chief nucleus without the neighboring secondary alcohol group. On oxidation it first yields *dehydrocholic acid*, $C_{24}H_{34}O_5$ (HAMMERSTEN) from which by electric reduction, SCHENCK obtained the reducto-dehydrocholic acid, $C_{24}H_{36}O_5$. On further oxidation *bilianic acid*, $C_{24}H_{34}O_8$ (CLEVE), is obtained, or, more correctly, according to LATSCHINOFF, LASSAR-COHN and PREGL, a mixture of bilianic and *isobilianic* acids discovered by LATSCHINOFF. On oxidation, bilianic acid yields *cilianic acid* (LASSAR-COHN), whose formula, according to PREGL,[4] is $C_{20}H_{28}O_8$.

The products formed on a more active oxidation are of great interest. If we discard the still somewhat problematic cholesterinic acid, we find in these products in the first place *choloidanic acid* which has also been called cholecamphoric acid and has the formula, $C_{18}H_{28}O_8$, according to PREGL. This acid, as well as the acid obtained by LETSCHE [5] on the oxidation of cholic acid and with the formula, $C_{19}H_{28}O_{10}$, have been obtained by PREGL [6] from the three most closely studied cholic acids,

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[1] Zeitschr. f. physiol. Chem., **61**.

[2] The important researches of Strecker on the bile-acids may be found in Annal. d. Chem. u. Pharm., **65**, **67**, and **70**; Mylius, Ber. d. deutsch. chem. Gesellsch., **19**.

[3] *Ibid.*, 39.

[4] Hammarsten, Ber. d. deutsch. chem. Gesellsch., **14**; Schenck, *ibid.*, **68** and **69**; Cleve, Bull. Soc. chim., **35**; Latschinoff, Ber. d. d. chem. Gesellsch., **15**; Lassar-Cohn, Ber. d. d. chem. Gesellsch., **32**; Pregl, Wein. Sitzungsber., **111**, 1902.

[5] Zeitschr. f. physiol. Chem., **61**.

[6] *Ibid.*, **65**.

namely from cholic acid, choleic acid and desoxycholic acid, and these three acids are identically constructed in regard to their 19 carbon atoms.

The choloidanic acid is interesting in several respects. PANZER[1] has obtained from it by distillation with soda-lime, a hydrocarbon, $C_{11}H_{16}$, a homologue of benzene, and on the oxidation of the cholic acid he has obtained an acid with the formula, $C_8O_{12}O_8$, which he considers as an oxyhexahydro-benzene-1-4-dicarboxylic acid and from which he obtained paraoxybenzaldehyde. PREGL has obtained from choloidanic acid, by heating, *pyrocholoidanic acid*, $C_{15}H_{20}O_4$ which he considers as parabenzoic acid *d*-methyl-*n*-capric acid, and is produced from the hexahydrobenzene derivative by total dehydrogenation of a benzene derivative.

v. FÜRTH and collaborators have investigated the products obtained on the dry distillation of cholic acid at ordinary pressure, and WIELAND and WEIL[2] on such distillation in vacuum. In the first case chiefly hydrocarbons with 12 to 17 carbon atoms were obtained, and in the second instance chiefly an unsaturated acid, $C_{24}H_{34}O_2$, was obtained, and in both cases these products and their double bindings have been carefully investigated. We must wait for further developments in these investigations before we attempt to draw any positive conclusions from them.

From the investigations on the cholic acids carried out thus far we are not able to draw any positive conclusions on their constitution, but that they are derivatives of hexahydrobenzene, is very probable for several reasons.

Cholic acid crystallizes partly in rhombic plates or prisms with one molecule of water, and partly in larger rhombic tetrahedra or octahedra with one molecule of alcohol of crystallization (MYLIUS). These crystals quickly become opaque and porcelain-white in the air. They are quite insoluble in water (in 4000 parts cold and 750 parts boiling), rather soluble in alcohol, but soluble with difficulty in ether. The amorphous cholic acid is less insoluble. The solutions have a bitter-sweetish taste. The crystals lose their alcohol of crystallization only after a lengthy heating to 100–120° C. The acid free from water and alcohol melts at 195–196° C. According to BONDI and MÜLLER the melting-point of the perfectly pure acid is 198° C. It forms a characteristic blue compound with iodine (MYLIUS). If finely powdered cholic acid is added to 25 per cent hydrochloric acid at the ordinary temperature, a beautiful violet-blue coloration gradually appears, and this color is permanent for some time and then becomes gradually green and yellow. The blue solution shows an absorption band in the neighborhood of the *D* line (HAMMARSTEN[3]).

The alkali salts are readily soluble in water, but when treated with a concentrated caustic or carbonated alkali solution, they may then be

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[1] Panzer, Zeitschr. f. physiol. Chem., **48** and **60**.

[2] v. Fürth with Link, Bioch. Zeitschr., **26**, with Ishihara, *ibid.*, **43**; Wieland and Weil, Zeitschr. f. physiol. Chem., **80**.

[3] Zeitschr. f. physiol. Chem., **61**.

separated as an oily mass which becomes crystalline on cooling. The alkali salts are not readily soluble in alcohol, and on the evaporation of the alcohol they may crystallize. The specific rotatory power of the sodium salt [1] is $(\alpha)_D = +30.61°$ (2.29 per cent concentration) to $+27.46°$ (7.59 per cent concentration). The watery solution of the alkali salts, when not too dilute, is precipitated immediately or after some time by lead acetate or by barium chloride. The barium salt crystallizes in fine, silky needles, and is rather insoluble in cold, but somewhat easily soluble in warm water. The barium salt, as well as the lead salt, which is insoluble in water, is soluble in warm alcohol.

Choleic Acid ($C_{25}H_{42}O_4$, LATSCHINOFF) is another cholic acid which, according to LASSAR-COHN,[2] has the formula, $C_{24}H_{40}O_4$. This acid, which occurs in varying but always small quantities in ox-bile, and also in gall-stones (H. FISCHER and P. MEYER [3]) yields *dehydrocholeic acid*, $C_{24}H_{34}O_4$, and then *cholanic acid*, $C_{24}H_{34}O_7$, and *isocholanic acid* on oxidation.

Choleic acid crystallizes when free from water in hexagonal vitreous prisms with pointed ends, melting at 185–187° C. The crystalline acid containing water melts at 135–140° C. (LATSCHINOFF). The acid dissolves in water with difficulty and is also relatively difficultly soluble in alcohol. It has an intensely bitter taste and gives the MYLIUS iodine reaction for cholic acid, and also the color reaction of cholic acid with hydrochloric acid. The specific rotation is $(\alpha)_D = +48.87°$ (VAHLEN). The barium salt which crystallizes from the hot alcoholic solution as spherical aggregations of radial needles is more difficultly soluble in water than the corresponding cholate.

Desoxycholic Acid, $C_{24}H_{40}O_4$, is the name given by MYLIUS [4] to a cholic acid isolated by him from putrid ox-bile, also in gall-stones (KÜSTER) and in fæces (FISCHER [5]), and which is formed from the cholic acid (on the putrefaction of the bile) by reduction. This last is still very improbable, and the investigations of EKBOM do not support such an assumption. On using perfectly pure cholic acid he was able to regain it almost quantitatively after the action of metallic sodium on the alcoholic solution of the acid, or of zinc and alkali. By treatment with zinc and glacial acetic acid a reaction took place, but the product was a mixture of mono- and diacetyl derivatives. The observation of PREGL

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[1] See Vahlen, Zeitschr. f. physiol. Chem., 21.

[2] Latschinoff, Ber. d. deutsch. chem. Gesellsch., 18 and 20; Lassar-Cohn, *ibid.*, 26, and Zeitschr. f. physiol. Chem., 17. See also Vahlen, Zeitschr. f. physiol. Chem., 23.

[3] Zeitschr. f. physiol. Chem., 76.

[4] Ber d. d. chem. Gesellsch, 19 and 20.

[5] Küster, Zeitschr. f. physiol. Chem., 69; H. Fischer, *ibid.*, 73.

that desoxycholic acid, like choleic acid, yields dehydrocholeic acid and cholanic acid as oxidation products, makes the formation of desoxycholic acid from cholic acid by reduction very improbable. The conclusion of LATSCHINOFF that both choleic and desoxycholic acids are identical, is not to be accepted on account of the different properties of the two acids, and as shown by LANGHELD and also found by HAMMARSTEN,[1] both acids can be detected in the same perfectly fresh ox-bile. PREGL[2] has given important proofs that we are here dealing with two different, probably, isomeric acids. He found that the two acids yielded dehydrocholeic acid on oxidation but that the dehydro-acid was not the same in both cases. The choleic acid yielded a dehydro-acid with a lower melting-point and a weaker specific rotation than the desoxycholic acid.

The desoxycholic acid crystallizes from glacial acetic acid in needles with 1 molecule acetic acid, having a melting-point of 144–145°. The melting-point of the acid crystallized from alcohol-ether is 153–155°, and for the anhydrous acid or crystallized from acetone it is 172–173°. It is soluble with difficulty in water, more readily soluble in alcohol, but somewhat less soluble in glacial acetic acid than choleic acid. It has an intensely bitter taste. The acid does not give a blue iodine compound, and no color reaction with hydrochloric acid. Its barium salt is soluble with difficulty in cold water, but dissolves in boiling alcohol and crystallizes on cooling.

The cholic acids are best prepared from ox-bile, which is boiled for 24 hours with 5–10 per cent caustic soda. The crude acid is precipitated by hydrochloric acid, dissolved in ammoniacal water and precipitated by $BaCl_2$. The precipitate contains essentially choleic and desoxycholic acids, while the filtrate contains a part of these and the chief part of the cholic acid. In regard to the further rather complicated method of separating the various acids, as also in regard to the many methods suggested for the preparation of the pure cholic acids, we must refer to more extensive hand-books.[3]

Fellic Acid, $C_{22}H_{40}O_4$ is a cholic acid, so called by SCHOTTEN, which he obtained from human bile, along with the ordinary acid. This acid is crystalline, is insoluble in water, and yields barium and magnesium salts, which are very insoluble. It does not respond to PETTENKOFER'S reaction easily and gives a more reddish-blue color. The existence of this acid is still doubtful.

The conjugate acids of human bile have not been sufficiently investigated. To all appearances human bile contains under different circum-

[1] Ekbom, Zeitschr. f. physiol. Chem., **50**; Pregl, Wien. Sitz-Ber. Bd., **111**, Math. Naturw. Kl., 1902; Latschinoff, Ber. d. d. Chem. Gesellsch., **20**; Langheld, *ibid.*, **41**; Hammarsten in Abderhalden's Handbuch d. bioch. Arbeitsmethoden Bd. **2**, 2.

[2] Zeitschr f. physiol. Chem., **65**.

[3] Abderhalden's Handbuch d. bioch. Arbeitsmethoden Bd. II. 2; also Pregl and Buchtala, Zeitschr. f. physiol. Chem., 74 and Schryver, Journ. of Physiol., **44**.

stances various conjugate bile-acids. In some cases the bile-salts of human bile are precipitated by $BaCl_2$ and in others not. According to the statements of LASSAR-COHN [1] three cholic acids may be prepared from human bile, namely, ordinary CHOLIC ACID, CHOLEIC ACID, and FELLIC ACID.

Lithofellic Acid, $C_{20}H_{36}O_4$, is the acid related to cholic acid which occurs in the oriental bezoar stones, which is insoluble in water, comparatively easily soluble in alcohol, but only slightly soluble in ether.[2]

Lithocholic Acid, $C_{24}H_{40}O_3$, is a cholic acid found by H. FISCHER [3] in gallstones. It melts at 184–186° and is tasteless.

The hyo-glycocholic and cheno-taurocholic acids, as well as the glycocholic acid of the bile of rodents, yield corresponding cholic acids. This also seems to be the case with the glycocholic acid of the hippopotamus-bile, which stands very close to the pig-bile (HAMMARSTEN [4]). In the polar bear a third cholic acid exists besides cholic and choleic acids. It is called *ursocholeic acid*, $C_{19}H_{30}O_4$ or $C_{18}H_{28}O_4$ (HAMMARSTEN [5]). Also in the bile of other animals (walrus, seal) HAMMARSTEN [6] has found special cholic acids, *phocæcholic acids*, of which one, the α-acid crystallizes from benzene or petroleum ether in six-sided thin plates which melt at 152–154° C. Its formula seems to be $C_{22}H_{36}O_5$. The other, β-phocæcholic acid has the formula $C_{24}H_{40}O_5$ and is isomeric with cholic acid. The *isocholic acid* melts at 220–222° C.

On boiling with acids, on putrefaction in the intestine, or on heating, cholic acids lose water and are converted into anhydrides, the so-called *dyslysins*. The dyslysin, $C_{24}H_{36}O_3$, corresponding to ordinary cholic acid, which occurs in fæces, is amorphous, insoluble in water and alkalies. *Choloidic acid*, $C_{24}H_{38}O_4$, is called the first anhydride or an intermediary product in the formation of dyslysin. On boiling dyslysins with caustic alkali they are reconverted into the corresponding cholic acids.

THE DETECTION OF BILE-ACIDS IN ANIMAL FLUIDS. To obtain the bile-acids pure so that PETTENKOFER'S test can be applied to them, the protein and fat must first be removed. The protein is removed by making the liquid first neutral and then adding a great excess of alcohol, so that the mixture contains at least 85 vols. per cent of water-free alcohol. Now filter, extract the precipitated protein with fresh alcohol, unite all filtrates, distil the alcohol, and evaporate to dryness. The residue is

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[1] Schotten, Zeitschr. f. physiol. Chem., 11; Lassar-Cohn, Ber. d. deutsch. chem. Gesellsch., 27.

[2] See Jünger and Klages, Ber. d. deutsch. chem. Gesellsch. 28 (older literature).

[3] Zeitschr. f. physiol. Chem., 78.

[4] *Ibid.*, 74.

[5] *Ibid.*, 86.

[6] *Ibid.*, 61 and 68.

completely exhausted with strong alcohol, filtered, and the alcohol entirely evaporated from the filtrate. The residue is extracted with ether and dissolved in water, and filtered if necessary, and the solution precipitated by basic lead acetate and ammonia. The washed precipitate is dissolved in boiling alcohol, filtered while warm, and a few drops of soda solution added. Then evaporate to dryness, extract the residue with absolute alcohol, filter, and add an excess of ether. The precipitate now formed may be used for PETTENKOFER'S test. It is not necessary to wait for cystallization; but one must not consider the crystals which form in the liquid as being positively crystallized bile. It is also possible for needles of alkali acetate to be formed. In this connection it must be remarked that a confusion with phosphatides, which also give PETTENKOFER'S reaction, is not excluded, and a further testing and separation are advisable.

Bile-pigments. The bile-coloring matters known thus far are relatively numerous, and in all probability there are still more of them. Most of the known bile-pigments are not found in the normal bile, but occur either in post-mortem bile or principally in the bile concrements. The pigments which occur under physiological conditions in human bile are the reddish-yellow *bilirubin*, the green *biliverdin*, and sometimes also *urobilin* (and *urobilinogen*) or a closely related pigment. The pigments found in gall-stones are (besides the *bilirubin* and *biliverdin*) *choleprasin*, *bilifuscin*, *biliprasin*, *bilihumin*, *bilicyanin* and (*choletelin?*). Besides these, others have been noticed in human and animal bile by various observers. The two above-mentioned physiological pigments, bilirubin and biliverdin, are those which serve to give the golden-yellow or orange-yellow or sometimes greenish color to the bile; or when, as is most frequently the case in ox-bile, the two pigments are present in the bile at the same time, they produce the different shades between reddish-brown and green.

Bilirubin. This pigment has the formula, $C_{16}H_{18}N_2O_3$, or according to ORNDORFF and TEEPLE and KÜSTER,[1] more correctly $C_{32}H_{36}N_4O_6$, and is designated by the names CHOLEPYRRHIN, BILIPHÆIN, BILIFULVIN, and HÆMATOIDIN. It occurs chiefly in the gall-stones as calcium bilirubin. Bilirubin is present in the liver-bile of all vertebrates, and in the bladder-bile especially in man and carnivora; sometimes, however, the latter may have a green bile when fasting or in a starving condition. It also occurs in the contents of the small intestine, in the blood serum of the horse, in old blood extravasations (as hæmatoidin), and in the urine and the yellow-colored tissue in icterus.

On reduction with sodium amalgam MALY obtained a reduction product, which he called *hydrobilirubin*, with the formula, $C_{32}H_{40}N_4O_7$,

[1] Orndorff and Teeple, Salkowski's Festschrift, Berlin, 1904; Küster, Zeitschr. f. Physiol. Chem., 59.

and which shows great similarity to the urinary pigment, *urobilin*, as well as to *stercobilin* found in the contents of the intestine (MASIUS and VANLAIR [1]). The reduction products have been carefully investigated by H. FISCHER and then by PAUL MEYER and F. MEYER-BETZ. They have found that hydrobilirubin is a mixture of bodies, among which there is one which forms at least one-half and therefore, called *hemibilirubin*, gives colorless crystals, and according to FISCHER and MEYER-BETZ is identical with the urobilinogen of the urine. The formula of this body is, $C_{32}H_{44}N_4O_6$ or $C_{33}H_{44}N_4O_6$. The other body is amorphous but in properties and composition shows great similarity to the hemibilirubin. The analyses correspond closely to the formula, $C_{32}H_{40}N_4O_6$. This body as well as the hemibilirubin yields hæmatinic acid and methylethylmaleic imide on oxidation. As KÜSTER [2] first showed, bilirubin yields hæmatinic acid as oxidation product. It does not on the contrary yield methylethyl maleic imide.

PILOTY and THANNHAUSER [3] obtained *bilinic* acid, $C_{17}H_{26}N_2O_3$ from bilirubin on reduction with hydriodic acid and iodophosphonium. This acid corresponded to the hæmatopyrrolidine carboxylic acid obtained from hæmatoporphyrin. This bilinic acid is identical with the bilirubinic acid described below and hence has this name.[4] They also obtained an isomeric acid to phonopyrrolic acid, the *isophonopyrrol carboxylic acid* and in the potash fusion they found partly a dimethyl- and partly a trimethylpyrrol. From bilinic acid they later obtained on mild oxidation an intensely yellow colored acid, the *dehydrobilinic acid.*

From bilirubin and hemibilirubin, on heating with sodium methylate, H. FISCHER and RÖSE [5] have obtained 2, 4, 5- *trimethylpyrrol-3-propionic acid* which was previously obtained by H. FISCHER and BARTHOLOMÄUS from phonopyrrolcarboxylic acid. From bilirubinic acid on the contrary, with the same procedure they did not obtain this acid but another, *xanthobilirubinic acid*, $C_{17}H_{22}N_2O_3$, which is probably identical with dehydrobilinic acid, and which contains two atoms of hydrogen less than bilirubinic acid, and which can be retransformed into the latter by glacial acetic acid and hydriodic acid. As bilirubin, as well as hemibilirubin, yields xanthobilirubinic acid as a side product with sodium methylate, these experimenters consider this as a proof that the bilirubinic acid con-

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[1] Maly, Wien. Sitzungsber., **57**, and Annal. d. Chem., **163**; Masius and Vanlair, Centralbl. f. d. med. Wissensch., 1871, 369.

[2] Hans Fischer, Zeitschr. f. physiol. Chem., **73**, with Paul Meyer, *ibid.*, **75**, with Meyer-Betz, *ibid.*, **75**; Küster, *ibid.*, **26** and Ber. d. d. chem. Gesellsch, **32** and **35**.

[3] Piloty and Thannhauser, Annal. d. chem. u. Pharm., **390** and Ber d. d. chem. Gesellsch., **45**.

[4] Piloty, Ber. d. d. chem. Gesellsch., **46**, 1000; H. Fischer, *ibid.*, **46**, 1574.

[5] Ber. d. d. chem. Gesellsch., **46**, 439.

figuration exists already formed in these two bodies, and that the above-mentioned tetrasubstituted acid, which is not obtained from bilirubinic acid, must come from a special third pyrrol nucleus in the bilirubin and hemibilirubin. Hæmatinic acid (KÜSTER from bilirubin) and methylethylmaleic imide (H. FISCHER and MEYER from hemibilirubin) have been obtained from the two other pyrrol nuclei. Hæmatinic acid as well as methylethylmaleic imide have also been obtained from bilirubinic acid.

FISCHER and RÖSE [1] have earlier obtained, from hemibilirubin as well as from the above-mentioned bodies and from bilirubin, by reduction with hydriodic acid, glacial acetic acid, a new crystalline acid, the *bilirubinic acid*, $C_{17}H_{24}N_2O_3$. This acid, to which PILOTY and THANNHAUSER'S bilinic acid stands in close relation, yields hæmatinic as well as methylethylmaleic imide on oxidation. By changing the method of reduction FISCHER and RÖSE [2] have obtained *cryptopyrrol* and isophonopyrrolcarboxylic acid from bilirubin. The bilirubinic acid also yielded the same products.

The close relation of the blood pigments to the bile pigments was first shown by KÜSTER when he obtained the two hæmatinic acids (as imide) as oxidation products of these. This close relation is further shown by the investigations given above although it is perhaps too early to draw positive conclusions in regard to the structure of the two groups of pigments and the differences existing between them.

Bilirubin is sometimes amorphous and sometimes crystalline. The amorphous bilirubin is a reddish-yellow or reddish-brown powder; the crystals have a reddish-yellow, reddish-brown, or more reddish color, and sometimes they have nearly the color of crystalline chromic acid. The crystals, which can easily be obtained by allowing a solution of bilirubin in chloroform to evaporate spontaneously, are reddish-yellow, rhombic plates, whose obtuse angles are often rounded. On crystallizing from hot dimethylaniline it forms, on cooling, broad columns with both ends sharply cut (KÜSTER [3]). On dissolving in chloroform both kinds of crystals are converted into long needles or whetstones.

Bilirubin is insoluble in water, behaves like an acid, and occurs in animal fluids as soluble alkali bilirubin. It is very slightly soluble in ether, benzene, carbon disulphide, amyl alcohol, fatty oils, and glycerin. It is somewhat more soluble in alcohol. In cold chloroform it dissolves with difficulty, and is much more readily soluble in warm chloroform. Its solubility varies, and supersaturated solutions are readily formed (ORNDORFF and TEEPLE). The varying solubility of bilirubin

[1] Zeitschr. f. physiol. Chem., **32**.

[2] Ber. d. d. chem. Gesellsch, **45**.

[3] *Ibid.*, **30** and **35**, and Zeitschr. f. physiol. Chem., **47**.

in chloroform depends, according to KÜSTER, on the fact that in its preparation, derivatives which are readily soluble and contain chlorine or other transformation products are formed, or perhaps the bilirubin goes over into polymeric modifications having different solubilities. In cold dimethylaniline it dissolves in the proportion of 1:100, and in hot dimethylaniline much more readily. Its solutions show no absorption-bands, but only a continuous absorption from the red to the violet end of the spectrum, and they have a decided yellow color, even on diluting greatly (1:500000), in a layer 1.5 cm. thick. The combinations of bilirubin with alkali are insoluble in chloroform, and the bilirubin in solution in chloroform can be removed from this solution by shaking with dilute alkali (differing from lutein). Solutions of bilirubin-alkali in water are precipitated by the soluble salts of the alkaline earths and also by metallic salts. If a dilute solution of alkali bilirubin in water is treated with an excess of ammonia and then with a zinc-chloride solution, the liquid is first colored deep orange and then gradually olive-brown and then green. This solution first gives a darkening of the violet and blue part of the spectrum, and then the bands of alkaline cholecyanin (see below), or at least the bands of this pigment in the red between *C* and *D*, close to *C*. This is a good reaction for bilirubin. The following reaction has been suggested by AUCHÉ[1]. Treat 5 cc. of an alcoholic solution of bilirubin (1:20000) which contains 1 drop of ammonia in 100 cc., with 5 to 6 drops of an alcoholic zinc acetate solution (1:1000) and then 1 drop alcoholic iodine solution (1:100) when a beautiful bluish-green coloration with a beautiful garnet-red fluorescence is obtained on shaking. The spectrum shows a dark band between *B* and *C*, and a pale band at *D*. If a few drops of hydrochloric acid are added to the solution the color becomes violet, the fluorescence disappears and the two JAFFÉ'S cholecyanin bands appear. This reaction is extremely delicate.

As EHRLICH first showed, bilirubin forms combinations with diazo compounds, which have been closely studied by PRÖSCHER, ORNDORFF and TEEPLE.[2] A test suggested by EHRLICH for bilirubin is based upon this behavior with sulphodiazobenzene.

If an alkaline solution of bilirubin be allowed to stand in contact with the air, it gradually absorbs oxygen, and green biliverdin is formed. This process is accelerated by warming. According to KÜSTER, in this case the alkali also has a splitting action upon the pigment, and among the products formed we find hæmatinic acid. Biliverdin is formed only

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[1] Compt. rend. soc. biol., **64**.

[2] Ehrlich, Zeitschr. f. anal. Chem., **23**; Pröscher, Zeitschr. f. physiol. Chem., **39**; Orndorff and Teeple, l. c.

from bilirubin by oxidation under special conditions (KÜSTER). A green coloring-matter similar in appearance is formed by the action of other reagents such as Cl, Br, and I. According to JOLLES,[1], biliverdin is produced by the use of HÜBL'S iodine solution, while according to others (THUDICHUM, MALY [2]) substitution products of bilirubin are formed.

GMELIN'S *Reaction for Bile-pigments.* If one carefully pours nitric acid, containing some nitrous acid, under an aqueous solution of alkali bilirubin, there is obtained a series of colored layers at the juncture of the two liquids in the following order from above downward: Green, blue, violet, red, and reddish-yellow. This color reaction, GMELIN'S test, is very delicate, and serves to detect the presence of one part bilirubin in 80,000 parts liquid. The green ring must never be absent; and also the reddish-violet must be present at the same time, otherwise the reaction may be confused with that for lutein, which gives a blue or greenish ring. The nitric acid must not contain too much nitrous acid, for then the reaction takes place too quickly and it does not become typical. Alcohol must not be present in the liquid, because, as is well known, it gives a play of colors, in green or blue, with the acid.

HAMMARSTEN'S *Reaction.* An acid is first prepared consisting of 1 vol. nitric acid and 19 vols. hydrochloric acid (each acid being about 25 per cent). One volume of this acid mixture, which can be kept for at least a year, is, when it has become yellow by standing, mixed with 4 vols. alcohol. If a drop of bilirubin solution is added to a few cubic centimeters of this colorless mixture a permanent beautiful green color is obtained immediately. On the further addition of the acid mixture to the green liquid all the colors of GMELIN'S scale, as far as choletelin, can be produced consecutively.

HUPPERT'S *Reaction.* If a solution of alkali bilirubin is treated with milk of lime or with calcium chloride and ammonia, a precipitate is produced consisting of calcium bilirubin. If this moist precipitate, which has been washed with water, is placed in a test-tube and the tube half filled with alcohol which has been acidified with hydrochloric acid, and heated to boiling for some time, the liquid becomes emerald-green or bluish-green in color.

In regard to the modifications of GMELIN'S test and certain other reactions for bile-pigments, see Chapter XIV (Urine).

That the characteristic play of colors in GMELIN'S test is the result of an oxidation is generally admitted. The first oxidation step is the

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[1] Küster, Ber. d. d. chem. Gesellsch., **35** and **59**; Jolles, Journ. f .prakt. Chem. (N.F.), **59**, and Pflüger's Arch., **75**.

[2] Thudichum, Journ. of Chem. Soc. (2), **18**, and Journ. f. prakt. Chem. (N.F.), **53**; Maly, Wien. Sitzungsber., **72**.

green biliverdin. Then follows a blue coloring-matter which HEINSIUS and CAMPBELL call *bilicyanin*, and STOKVIS calls *cholecyanin*, and which shows a characteristic absorption-spectrum. The neutral solutions of this coloring-matter are, according to STOKVIS, bluish-green or steel-blue with a beautiful blue fluorescence. The alkaline solutions are green and have no marked fluorescence, and show three absorption-bands: one, sharp and dark, in the red between *C* and *D*, nearer to *C*; a second, less well defined, covering *D*; and a third between *E* and *F*, near *E*. The strongly acid solutions are violet-blue and show two bands, described by JAFFÉ between the lines *C* and *E*, separated from each other by a narrow space near *D*. A third band between *b* and *F* is seen with difficulty. The next oxidation step after these blue coloring-matters is a red pigment, and lastly a yellowish-brown pigment, called *choletelin*, by MALY, which in neutral alcoholic solutions does not give any absorption-spectrum, but in acid solution gives a band between *b* and *F*. On oxidizing cholecyanin with lead peroxide, STOKVIS [1] obtained a product which he calls choletelin, which is quite similar to urinary urobilin, to be discussed later.

Bilirubin is best prepared from gall-stones of oxen, these concretions being very rich in calcium bilirubin. The finely powdered concrement is first exhausted with ether and then with boiling water, so as to remove the cholesterin and bile-acids. In order to remove the mineral constituents it is better to use 10 per cent acetic acid instead of hydrochloric acid (KÜSTER [2]). A green pigment is now removed by extraction with alcohol, and the choleprasin is extracted with hot glacial acetic acid. After washing with water it is dried, and extracted repeatedly with boiling chloroform. The bilirubin separates from the chloroform as crusts, which are treated once or twice in the above manner. It is then extracted with alcohol and precipitated from its chloroform solution by alcohol, or crystallized from boiling dimethylaniline. Further details are given by KÜSTER.[3]

The quantitative estimation of bilirubin may be made by the spectrophotometric method, according to the steps suggested for the blood-coloring matters.[4]

Biliverdin, $C_{16}H_{18}N_2O_4$ or $C_{32}H_{36}N_4O_8$. This body, which is formed by the oxidation of bilirubin, occurs in the bile of many animals, in vomited matter, in the placenta of the bitch (?), in the shells of birds'

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[1] Heinsius and Campbell, Pflüger's Arch., 4; Stokvis, Centralbl. f. med. Wissensch., 1872, 785; *ibid.*, 1873, 211 and 449; Jaffé, *ibid.*, 1868; Maly, Wien. Sitzungsber., 59.

[2] Zeitschr. f. physiol. Chem., 47.

[3] *Ibid.*, 59.

[4] See also Herzfeld, Zeitschr. f. physiol. Chem., 77 and 78.

eggs, in the urine in icterus, and sometimes in gall-stones, although in very small quantities.

Biliverdin is amorphous; at least it has not been obtained in well-defined crystals. It is insoluble in water, ether, and chloroform (this is true at least for the artificially prepared biliverdin) but is soluble in alcohol or glacial acetic acid, showing a beautiful green color. It is dissolved by alkalies, giving a brownish-green color, and this solution is precipitated by acids, as well as by calcium, barium, and lead salts. Biliverdin gives HUPPERT'S, GMELIN'S, and HAMMARSTEN'S reactions, commencing with the blue color. It is converted into hydrobilirubin by nascent hydrogen. On allowing the green bile to stand, also by the action of ammonium sulphide, the biliverdin may be reduced to bilirubin (HAYCRAFT and SCOFIELD [1]).

Biliverdin is most simply prepared by allowing a thin layer of an alkaline solution of bilirubin to stand exposed to the air in a dish until the color is brownish-green. The solution is then precipitated by hydrochloric acid, the precipitate washed with water until no HCl reaction is obtained, then dissolved in alcohol and the pigment again separated by the addition of water. Any contaminating bilirubin may be removed by means of chloroform. KÜSTER has shown that the biliverdin is only formed by the oxygen of the air from bilirubin under certain conditions: The presence of 2 molecules caustic alkali with the addition of water so that the solution contains 0.2 per cent and, a temperature not above 5° C. HUGOUNENQ and DOYON [2] prepared biliverdin from bilirubin by the action of sodium peroxide and a little hydrochloric acid.

Choleprasin is a green pigment isolated by KÜSTER [3] from gall-stones, which is soluble in glacial acetic acid but insoluble in alcohol. It differs from the other bile-pigments by containing sulphur. On distillation with zinc powder it gives the pyrrol reaction, and on oxidation with chromic acid, KÜSTER could not observe any formation of hæmatinic acid.

Bilifuscin, so named by STÄDELER,[4] is an amorphous brown pigment soluble in alcohol and alkalies, almost insoluble in water and ether, and soluble with great difficulty in chloroform (when bilirubin is not present at the same time). Pure bilifuscin does not give GMELIN'S reaction. This is also true for the bilifuscin prepared by V. ZUMBUSCH,[5] which is more like a humin substance, and the formula of which is, $C_{64}H_{96}N_7O_{14}$. Bilifuscin has been found in gall-stones. *Biliprasin* is a green pigment prepared by STÄDELER from gall-stones, and is generally considered as a mixture of biliverdin and bilirubin. DASTRE and FLORESCO,[6] on the

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[1] Centralbl. f. Physiol., **3**, 222, and Zeitschr. f. physiol. Chem., **14**.

[2] Hugounenq et Doyon, Arch. de Phyisol. (5), 8; Küster, Zeitschr. f. physiol. Chem., **59**.

[3] Zeitschr. f. physiol. Chem., **47**.

[4] Cited from Hoppe-Seyler, Physiol. u. Path. chem. Analyse, 6. Aufl., p. 225.

[5] Zeitschr. f. physiol. Chem., **31**.

[6] Arch. de Physiol. (5), **9**.

contrary, consider biliprasin as an intermediate step between bilirubin and biliverdin. According to them it occurs as a physiological pigment in the bladder-bile of several animals, and is derived from bilirubin by oxidation. This oxidation is brought about by an oxidative ferment existing in the bile. *Bilihumin* is the name given by STÄDELER to that brownish amorphous residue which is left after extracting gall-stones, with chloroform alcohol, and ether. It does not give GMELIN'S test. *Bilicyanin* is also found in human gall-stones (HEINSIUS and CAMPBELL). *Cholohæmatin*, so-called by MACMUNN, is a pigment often occurring in sheep- and ox-bile and characterized by four absorption-bands, which is formed from hæmatin by the action of sodium amalgam. In the dried condition, as when obtained by the evaporation of the chloroform solution, it is green, and in alcoholic solution olive-brown. This pigment, which has also been found by HAMMARSTEN in the bile from the musk-ox and hippopotamus, is, according to MARCHLEWSKI, identical with the crystalline *bilipurpurin* isolated by LOEBISCH and FISCHLER from ox-bile. This latter pigment, according to MARCHLEWSKI, is not a bile-pigment, but *phylloerythrin*, a transformation product of chlorophyll. Phylloerythrin has been detected by MARCHLEWSKI [1] in the excrement of cows fed on green grass.

GMELIN'S and HUPPERT'S reactions are generally used to detect the presence of bile-pigments in animal fluids or tissues. The first, as a rule, can be performed directly, and the presence of proteins does not interfere with it, but, on the contrary, it brings out the play of colors more strikingly. If blood-coloring matters are present at the same time, the bile-coloring matters are first precipitated by the addition of sodium phosphate and milk of lime. This precipitate containing the bile-pigments may be used directly in HUPPERT'S reaction, or a little of the precipitate may be dissolved in HAMMARSTEN'S reagent. Bilirubin is detected in blood, according to HEDENIUS, by precipitating the proteins with alcohol, filtering and acidifying the filtrate with hydrochloric or sulphuric acid, and boiling. The liquid becomes of a greenish color. Serum and serous fluids may be boiled directly with a little acid after the addition of alcohol. According to OBERMEYER and POPPER [2] the alcoholic filtrate from the protein precipitation can be tested with an alcoholic solution of iodine or ferric chloride.

Besides the bile-acids and the bile-pigments, there occur in the bile also *cholesterin*, *lecithin*, *jecorin* or other *phosphatides* (HAMMARSTEN), *palmitin*, *stearin*, *olein*, *myristic acid* (LASSAR-COHN[3]), *soaps*, *ethereal sulphuric acids*, *conjugated glucuronates*, *diastatic* and *proteolytic enzymes*, *oxidases* and *catalases*. *Choline*, and *glycerophosphoric acid*, when they are present, may be considered as decomposition products of lecithin. *Urea* occurs, though only in traces, as a physiological constituent of human,

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[1] MacMunn, Journ. of Physiol., 6; Loebisch and Fischler, Wien. Sitzungsber., 112 (1903); Marchlewski, Zeitschr. f. physiol. Chem., 41, 43, and 45; Hammarsten, *ibid.*, 43, and investigations not published.

[2] Hedenius Upsala Läkaref. Förh., 29 and Maly's Jahresber., 24; Obermeyer and Popper, Wien. med. Wochenschr., 60.

[3] Zeitschr. f. physiol. Chem., 17; Hammarsten, *ibid.*, 32, 36 and 43.

ox-, and dog-bile. Urea occurs in the bile of the shark and ray in such large quantities that it forms one of the chief constituents of the bile.[1] The *mineral constituents* of the bile are, besides the alkalies, to which the bile-acids are united, sodium and potassium chloride, calcium and magnesium phosphate, and iron—0.04–0.115 p. m. in human bile, chiefly combined with phosphoric acid (YOUNG[2]). Traces of copper are habitually present, and traces of zinc are often found. Sulphates are entirely absent, or occur only in very small amounts.

The quantity of iron in the bile varies greatly. According to NOVI it is dependent upon the kind of food, and in dogs it is lowest with a bread diet and highest with a meat diet. According to DASTRE this is not the case. The quantity of iron in the bile varies even though a constant diet is maintained, and the variation is dependent upon the formation and destruction of blood. According to BECCARI[3] the iron does not disappear from the bile in inanition, and the percentage shows no constant diminution. The question as to the extent of elimination by the bile of the iron introduced into the body has received various answers. There is no doubt that the liver has the property of collecting and retaining iron, as well as other metals, from the blood. Certain investigators, such as NOVI and KUNKEL, are of the opinion that the iron introduced and transitorily retained in the liver is eliminated by the bile, while others, such as HAMBURGER, GOTTLIEB, and ANSELM,[4] deny any such elimination of iron by the bile.

Quantitative Composition of the Bile. Complete analyses of human bile have been made by HOPPE-SEYLER and his pupils. The bile was removed from the gall-bladder of cadavers, hence these analyses can be of little interest. Older and less complete analyses of perfectly fresh human bile have been made by FRERICHS and v. GORUP-BESANEZ.[5] The bile analyzed by them was from perfectly healthy persons who had been executed or accidentally killed. The two analyses of FRERICHS are, respectively, of (I) an 18-year-old and (II) a 22-year-old male. The analyses of v. GORUP-BESANEZ are of (I) a man of 49 and (II) a woman of 29. The results are, as usual, in parts per 1000.

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[1] Hammarsten, *ibid.*, 24.

[2] Journ. of Anat. and Physiol., 5, 158.

[3] Novi, see Maly's Jahresber., 20; Dastre, Arch. de Physiol. (5), 3; Beccari, Arch. ital. de Biol., 28.

[4] Kunkel, Pflüger's Arch., 14; Hamburger, Zeitschr. f. physiol. Chem., 2 and 4; Gottlieb, *ibid.*, 15; Anselm, "Ueber die Eisenausscheidung der Galle," Inaug.-Diss. Dorpat, 1891. See also the works cited in footnote 3, p. 339.

[5] See Hoppe-Seyler Physiol. Chem., 301; Socoloff, Pflüger's Arch., 12; Trifanowski, *ibid.*, 9; Frerichs in Hoppe-Seyler's Physiol. Chem., 299; v. Gorup-Besanez, *ibid.*

	Frerichs.		v. Gorup-Besanez.	
	I.	II.	I.	II.
Water	860.0	859.2	822.7	898.1
Solids	140.0	140.8	177.3	101.9
Biliary salts	72.2	91.4	107.9	56.5
Mucus and pigments	26.6	29.8	22.1	14.5
Cholesterin	1.6	2.6	47.3	30.9
Fat	3.2	9.2		
Inorganic substances	6.5	7.7	10.8	6.2

Human liver-bile is poorer in solids than the bladder-bile. In several cases it contained only 12–18 p. m. solids, but the bile in these cases is hardly to be considered as normal. Jacobsen found 22.4–22.8 p. m. solids in a specimen of bile. Hammarsten, who had occasion to analyze the liver-bile in seven cases of biliary fistula, has often found 25–28 p. m. solids. In a case of a corpulent woman the quantity of solids in the liver-bile varied between 30.10–38.6 p. m. in ten days. Brand [1] observed still higher figures, more than 40 p. m., in two cases. This investigator suggests that the bile from an imperfect fistula, when it is partly absorbed, is richer in solids than when it comes from a perfect fistula.

The molecular concentration of human bile, according to Brand, Bonanni, and Strauss,[2] is generally identical with that of the blood, although the amount of water and solids varies. The freezing-point varies only between $-0.54°$ and $-0.58°$. This constancy of the osmotic pressure is explained by the fact that in concentrated biles with larger amounts of organic substances (with larger molecules) the amount of inorganic salts is lower.[3]

Human bile, sometimes, but not always, contains sulphur in an ethereal sulphuric-acid-like combination (Hammarsten, Oerum, Brand). The quantity of such sulphur may even amount to $\frac{1}{4}$–$\frac{1}{3}$ of the total sulphur. We do not know the nature of these ethereal sulphuric acids. According to Oerum [4] they are not precipitated by lead acetate, but are precipitated by basic lead acetate, especially with ammonia. Human bile is habitually richer in glycocholic than in taurocholic acid. In six cases of liver-bile analyzed by Hammarsten the relation of taurocholic to glycocholic acid varied between 1:2.07 and 1:14.36. The bile analyzed by Jacobsen contained no taurocholic acid.

As an example of the composition of human liver-bile the following

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[1] Jacobsen, Ber. d. deutsch. chem. Gesellsch., 6; Hammarsten, Nova Acta Reg. Soc. Scient. Upsala, 16; Brand, Pflüger's Arch., 90.

[2] Brand, l. c., Bonanni, Biochem. Centralbl., 1; Strauss, Berl. klin. Wochenschr., 1903.

[3] See Brand, l. c.; Hammarsten, l. c.

[4] Skand. Archiv. f. Physiol., 16.

results of three analyses made by HAMMARSTEN are given. The results are calculated in parts per 1000:[1]

Solids	25.200	35.260	25.400
Water	974.800	964.740	974.600
Mucin and pigments	5.290	4.290	5.150
Bile-salts	9.310	18.240	9.040
Taurocholate	3.034	2.079	2.180
Glycocholate	6.276	16.161	6.860
Fatty acids from soaps	1.230	1.360	1.010
Cholesterin	0.630	1.600	1.500
Lecithin	0.220	0.574	0.650
Fat		0.956	0.610
Soluble salts	8.070	6.760	7.250
Insoluble salts	0.250	0.490	0.210

Among the mineral constituents the chlorine and sodium occur to the greatest extent. The relation between potassium and sodium varies considerably in different samples, Sulphuric acid and phosphoric acid occur only in very small quantities.

BAGINSKY and SOMMERFELD[2] found true mucin, mixed with some nucleoalbumin, in the bladder-bile of children. The bile contained on an average 896.5 p. m. water; 103.5 p. m. solids; 20 p. m. mucin; 9.1 p. m. mineral substances; 25.2 p. m. bile-salts (of which 16.3 p. m. were glycocholate and 8.9 p. m. taurocholate); 3.4 p. m. cholesterin; 6 p. m. lecithin; 6.7 p. m. fat, and 2.8 p. m. leucine.[3]

The quantity of pigment in human bile is, according to NOËL-PATON, 0.4–1.3 p. m. (in a case of biliary fistula). The method used in determining the pigments in this case was not quite trustworthy. Accurate results for dog-bile obtained by spectrophotometric methods are on record. According to STADELMANN[4] dog-bile contains on an average 0.6–0.7 p. m. bilirubin. At the most only 7 milligrams of pigment are secreted per kilo of body in the twenty-four hours.

In animals the relative proportion of the two acids varies considerably. It has been found, on determining the amount of sulphur, that, so far as the experiments have gone, taurocholic acid is the prevailing acid in carnivorous mammals, birds, snakes, and fishes. Among the herbivora, sheep and goats have a predominance of taurocholic acid in the bile. Ox-bile sometimes contains taurocholic acid in excess, in other cases glycocholic acid predominates, and in a few cases the latter occurs almost alone. The bile of the rabbit, hare, kangaroo,

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[1] Recent quantitative analyses may be found in Brand, l. c.; v. Zeynek, Wien. klin. Wochenschr., 1899; Bonanni, l. c.

[2] Verhandl. d. physiol. Gesellsch. zu Berlin, 189–95.

[3] Analyses of bile from children may be found in Heptner, Maly's Jahresber., 30.

[4] Noël-Paton, Rep. Lab. Roy. Soc. Coll. Phys. Edinburgh, 3; Stadelmann, Der Icterus.

hippopotamus, and orang-utang (HAMMARSTEN [1]) contains, like the bile of the pig, almost exclusively glycocholic acid. A distinct influence on the relative amounts of the two bile-acids exerted by differences in diet has not been detected. RITTER [2] claims to have found a decrease in the quantity of taurocholic acid in calves when they pass from the milk to the vegetable diet.

In the above-mentioned calculation of the taurocholic acid from the quantity of sulphur in the bile-salt, it must be remarked that no definite conclusion can be drawn from such a determination, since it is known that other kinds of bile (e. g., human and shark bile) contain sulphur in compounds other than taurocholic acid.[3]

The phosphorized constituents of bile are not well known; nevertheless, there is no doubt that bile contains other phosphatides besides lecithin (HAMMARSTEN). These phosphatides are in part precipitated in the precipitation of the bile-salts and they in part keep the bile-salts in solution, preventing their complete precipitation, and hence they have a double disturbing action in the quantitative analysis of bile. Those biles richest in phosphatides, so far as known, are the following, in the order of their amount: Polar bear, man (in special cases), dog, black bear, orang-utang. The bile of certain fishes contains but little phosphatides (HAMMARSTEN [4]).

The cholesterin, which, according to several investigators, originates not only from the liver but also from the biliary passages, occurs in larger quantities in the bladder-bile than in the liver-bile, and is present to a greater extent in the non-filtered than in the filtered bile (DOYON and DUFOURT). The quantity seems to be very variable and in patients with bile fistulas BACMEISTER [5] found 0.24–0.59 p. m. The *gases* of the bile consist of a large quantity of carbon dioxide, which increases with the amount of alkalies, only traces of oxygen, and a very small quantity of nitrogen.

Little is known in regard to the *composition of the bile in disease*. The quantity of *urea* is found to be considerably increased in uræmia. *Leucine* and *tyrosine* are observed in acute yellow atrophy of the liver and in typhoid. Traces of *albumin* (without regard to nucleoalbumin) have several times been found in the human bile. The so-called *pigmentary acholia*, or the secretion of a bile containing bile-acids but no bile-pigments, has also been repeatedly noticed. In all such cases observed by RITTER he found a fatty degeneration of the liver-cells, in return for which, even in excessive fatty infiltration, a normal bile containing pigments was secreted. The secretion of a bile nearly free from bile-acids has been

[1] See Ergebnisse der Physiol., 4.

[2] Cited from Maly's Jahresber., 6, 195.

[3] Hammarsten, Zeitschr. f. physiol. Chem., 32, and Ergebnisse, der Physiol., 4.

[4] Zeitschr. f. physiol. Chem., 36, and Ergebnisse der Physiol., 4.

[5] Doyon and Dufort, Arch. de Physiol. (5), 8; Bacmeister, Bioch. Zeitschr., 26.

observed by HOPPE-SEYLER [1] in amyloid degeneration of the liver. In animals, dogs, and especially rabbits, it has been observed that the blood-pigments pass into the bile in poisoning and in other conditions, causing a destruction of the blood-corpuscles, as also after intravenous hæmoglobin injection (WERTHEIMER and MEYER, FILEHNE, STERN [2]). Albumin can pass into the bile after the intravenous injection of a foreign protein (casein) (GÜRBER and HALLAUER), as well as after poisoning with phosphorus or arsenic (PILZECKER), or after the irritation of the liver by the introduction of ethyl alcohol or amyl alcohol (BRAUER). Sugar occurs in bile only in exceptional cases.[3]

The physiological secretion of the gall-bladder in man is, according to WAHLGREN [4] a viscous, alkaline fluid with 11.24–19.63 p. m. solids. The mucilaginous properties are not due to mucin, but to a phosphorized protein substance (nucleoalbumin or nucleoprotein).

Instead of bile there is sometimes found in the gall-bladder under pathological conditions a more or less viscous, thready, colorless fluid which contains pseudo-mucins or other peculiar protein substances.[5]

Chemical Formation of the Bile. The first question to be answered is the following: Do the specific constituents of the bile, the bile-acids and bile-pigments originate in the liver; and if this is the case, do they come from this organ alone, or are they also formed elsewhere?

The investigations of the blood, and especially the comparative investigations of the blood of the portal and hepatic veins under normal conditions, have not given any answer to this question. To decide this, therefore, it is necessary to extirpate the liver of animals or to isolate it from the circulation. If the bile constituents are not formed in the liver, or at least not alone in this organ, but are eliminated only from the blood, then, after the extirpation or removal of the liver from the circulation, an accumulation of the bile constituents is to be expected in the blood and tissues. If the bile constituents, on the contrary, are formed exclusively in the liver, then the above operation naturally would give no such result. If the ductus choledochus is tied, then the bile constituents will be collected in the blood or tissues whether they are formed in the liver or elsewhere.

From these principles KÖBNER has tried to demonstrate by experiments on frogs that the *bile-acids* are produced exclusively in the liver. While he was unable to detect any bile-acids in the blood and tissues of

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[1] Ritter, Compt. Rend., 74, and Journ. de l'anat. et de la physiol. (Robin), 1872; Hoppe-Seyler, Physiol. Chem., 317.

[2] Wertheimer and Meyer, Compt. Rend., 108; Filehne, Virchow's Arch., 121; Stern, *ibid.*, 123.

[3] Gürber, and Hallauer, Zeitschr. f. Biologie, 45; Pilzecker, Zeitschr. f. physiol. Chem., 41; Brauer, *ibid.*, 40.

[4] See Maly's Jahresber., 32.

[5] Winternitz, Zeitschr. f. physiol. Chem., 21; Sollmann, Amer. Medicine, 5 (1903).

these animals after extirpation of the liver, he was able to discover them on tying the ductus choledochus. The investigations of LUDWIG and FLEISCHL [1] show that in the dog the bile-acids originate in the liver alone. After tying the ductus choledochus, they observed that the bile constituents were absorbed by the lymphatic vessels of the liver and passed into the blood through the thoracic duct. Bile-acids could be detected in the blood after such an operation, while they could not be detected in the normal blood. But when the common bile and thoracic ducts were both tied at the same time, then not the least trace of bile-acids could be detected in the blood, while if they are also formed in other organs and tissues they should have been present.

From earlier reports of CLOEZ and VULPIAN, as well as VIRCHOW, the bile-acids also occur in the suprarenal capsule. These claims have not been confirmed by later investigations of STADELMANN and BEIER.[2] At the present time there is no ground for supposing that the bile-acids are formed elsewhere than in the liver.

It has been undoubtedly proved that the *bile-pigments* may be formed in other organs besides the liver, for, as is generally admitted, the coloring-matter hæmatoidin, which occurs in old blood extravasations, is identical with the bile-pigment bilirubin (see page 301). LATSCHENBERGER [3] also observed in horses, under pathological conditions, a formation of bile-pigments from the blood-coloring matters in the tissues. The occurrence of bile-pigments in the placenta also seems to depend on their formation in that organ, while the occurrence of small quantities of bile-pigments in the blood-serum of certain animals probably depends on an absorption of these substances.

Although the bile-pigments may be formed in other organs besides the liver, still it is of first importance to know what bearing this organ has on the elimination and formation of bile-pigments. In this regard it must be recalled that the liver is an excretory organ for the bile-pigments circulating in the blood. TARCHANOFF observed in a dog with biliary fistula, that intravenous injection of bilirubin causes a very considerable increase in the bile-pigments eliminated. This statement has been later confirmed by the investigations of VOSSIUS.[4]

Numerous experiments have been made to decide the question whether the bile-pigments are only eliminated by the liver, or whether they are also formed therein. By experimenting on pigeons, STERN was able

[1] Köbner, see Heidenhain, Physiologie der Absonderungsvorgänge, in Hermann's Handbuch, 5; Fleischl, Arbeiten aus der physiol. Anstalt zu Leipzig, Jahrgang, 9.

[2] Zeitschr. f. physiol. Chem., 18, in which the older literature may be found.

[3] See Maly's Jahresber., 16, and Monatshefte f. Chem., 9.

[4] Tarchanoff, Pflüger's Arch., 9; Vossius, cited from Stadelmann, Der Icterus.

to detect bile-pigments in the blood-serum five hours after tying the biliary passages alone, while after tying all the vessels of the liver and also the biliary passages, no bile-pigments could be detected either in the blood or the tissues of the animal, which was killed 10–24 hours after the operation. MINKOWSKI and NAUNYN [1] also found that poisoning with arseniureted hydrogen produces a liberal formation of bile-pigments, and the secretion, after a short time, of a urine rich in biliverdin in previously healthy geese. In geese with extirpated livers this does not occur.

With experiments on dogs, WHIPPLE and HOOPER [2] found after intravenous injection of blood-corpuscles of the same animal hæmolyzed with water, that a transformation of the hæmoglobin into bile-pigments occurred with the same rapidity in normal animals as with animals with Eck fistulas, or with such a fistula and the hepatic artery ligatured. The formation of bile-pigments also occurred on removing the liver, spleen and abdomen from the circulation, as well as by circulation through the head and thorax. A transformation of hæmoglobin into bile-pigments, at least in dogs, can take place easily without the medium of the liver and these experimenters suggest the possibility that the endothelial cells are here active.

No such experiments can be carried out on mammalia, as they do not live long enough after the operation; still there is no doubt that this organ is the chief seat of the formation of bile-pigments under physiological conditions.

In regard to the materials from which the bile-acids are produced, it may be said with certainty that the two components, glycocoll and taurine, which are both nitrogenized, are formed from the protein bodies. The close relation of taurine to the cystine group of the protein molecule has been especially shown by the investigations of FREIDMANN, (see Chapter III), and recently v. BERGMANN [3] has shown by feeding dogs with sodium cholate and cystine that the animal body can transform cystine into taurine, and that the taurine of the bile originates from the proteins of the food. In regard to the origin of the non-nitrogenized cholic acid, which was formally considered as originating from the fats, nothing is positively known; to all appearances it is from proteins.

The blood-coloring matters are considered as the mother-substances of the bile-pigments. If the identity of hæmatoidin and bilirubin was

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[1] Stern, Arch. f. exp. Path. u. Pharm., 19; Minkowski and Naunyn, *ibid.*, 21.

[2] Journ. of exp. Med., 17.

[3] Hofmeister's Beiträge, 4. See also Wohlgemuth, Zeitschr. f. physiol. Chem., 40.

settled beyond a doubt, then this view might be considered as proved. Independently, however, of this identity, which is not admitted by all investigators, the view that the bile-pigments are derived from the blood-coloring matters has strong arguments in its favor. It has been shown by several experimenters that a yellow or yellowish-red pigment can be formed from the blood-coloring matters, which gives GMELIN'S test, and which, though it may not form a complete bile-pigment, is at least a step in its formation (LATSCHENBERGER). The previously mentioned relationship between the blood and bile-pigments must be recalled, and the formation of bilirubin from the blood-pigments is shown, according to the unanimous observations of several investigators,[1] by the fact that the appearance of free hæmoglobin in the plasma, produced by the destruction of the red corpuscles by widely differing influences (see below) or by the injection of hæmoglobin solution, causes an increased formation of bile-pigments. The amount of pigments in the bile is not only considerably increased, but the bile-pigments may even pass into the urine under certain circumstances (icterus). After the injection of hæmoglobin solution into a dog either subcutaneously or in the peritoneal cavity, STADELMANN and GORODECKI[2] observed an increase of 61 per cent in the secretion of pigments by the bile, which lasted for more than twenty-four hours. Recently BRUSCH and YOSHIMOTO,[3] by quantitative estimations of the bile-pigments and urobilin in animals with bile fistulas with ligated ductus choledochus, have shown the increased formation of bile-pigments after the injection of known amounts of hæmatin, and in this manner further proved the genetic relationship between the bile-pigments and hæmatin.

If bilirubin, which contains no iron, is derived from hæmatin, which contains iron, then iron must be split off. The question in what form or combination the iron is split off is of special interest, and also whether it is eliminated by the bile. This latter does not seem to be the case, at least to any great extent. In 100 parts of bilirubin which are eliminated by the bile there are only 1.4–1.5 parts iron, according to KUNKEL, while 100 parts hæmatin contain about 9 parts iron. MINKOWSKI and BASERIN[4] also found that the abundant formation of bile-pigments occurring in poisoning by arseniureted hydrogen does not increase the quantity of iron in the bile. The quantity apparently does not seem to correspond with that in the decomposed blood-coloring matters. It follows from the

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[1] See Stadelmann, Der Icterus, etc., Stuttgart, 1891.

[2] See Stadelmann, *ibid.*

[3] Zeitschr. f. exp. Path. u. Therap., 8.

[4] Kunkel, Pflüger's Arch., 14; Minkowski and Baserin, Arch. f. exp. Path. u. Pharm., 28.

researches of several investigators[1] that the iron is, at least chiefly, retained by the liver as a ferruginous pigment or protein substance.

What relation does the formation of bile-acids bear to the formation of bile-pigments? Are these two chief constituents of the bile derived simultaneously from the same material, and can we detect a certain connection between the formation of bilirubin and bile-acids in the liver? The investigations of STADELMANN teach us that this is not the case. With increased formation of bile-pigments the amount of bile-acids is decreased, and the introduction of hæmoglobin into the liver strongly increases the formation of bilirubin, but simultaneously strongly decreases the production of bile-acids. According to STADELMANN the formation of bile-pigments and bile-acids is due to a special activity of the cells.

An absorption of bile from the liver, and the passage of the bile constituents into the blood and urine occurs in retarded discharge of the bile, and usually in different forms of *hepatogenic icterus*. But bile-pigments may also pass into the urine under other circumstances, especially when a solution or destruction of the red blood-corpuscles takes place in animals through injection of water or a solution of biliary salts, through poisoning by ether, chloroform, arseniureted hydrogen, phosphorus, or toluylenediamine, and in other cases. This also occurs in man in severe infectious diseases where the red blood-corpuscles are dissolved or destroyed. It has also been claimed many times that a transformation of blood-pigments into bile-pigments occurs elsewhere than in the liver, namely, in the blood. Such a belief has been made very improbable and in some of the above-mentioned cases, as after poisoning with phosphorus, toluylenediamine, and arseniureted hydrogen, it has been disproved by direct experiment.[2] In these cases we are also dealing with an abundant working up of the blood-pigments in the liver.

Bile Concretions.

The concrements which occur in the gall-bladder vary considerably in size, form, and number, and are of three kinds, depending upon the kind and nature of the bodies forming their principal mass. One group of gall-stones contains lime-pigment as chief constituent, another cholesterin, and the third calcium carbonate and phosphate. The concrements of the last-mentioned group occur very seldom in man. The so-called cholesterin-stones are those which occur most frequently in man, while

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[1] See Naunyn and Minkowski, Arch. f. exp. Path. u. Pharm., **21**; Latschenberger, l.c.; Neumann, Virchow's Arch., **111**, and the literature in footnote 2, p. 383.

[2] The literature belonging to this subject is found in Stadelmann, Der Icterus, etc., Stuttgart, 1891.

the lime-pigment stones are not found very often in man, but often in oxen.

The *pigment-stones* are generally not large in man, but in oxen and pigs they are sometimes found the size of a walnut or even larger. In most cases they consist principally of calcium-bilirubin with little or no biliverdin, and they also often contain very small amounts of cholic acids. Sometimes also small black or greenish-black, metallic-looking stones are found, which consist chiefly of bilifuscin along with biliverdin. Iron and copper seem to be regular constituents of pigment-stones. Manganese and zinc have also been found in a few cases. The pigment-stones are generally heavier than water.

The *cholesterin-stones*, whose size, form, color, and structure may vary greatly, are often lighter than water. The fractured surface is radiated, crystalline, and frequently shows crystalline, concentric layers. The cleavage fracture is waxy in appearance, and the fractured surface when rubbed by the finger-nail also becomes like wax. By rubbing against each other in the gall-bladder they often become faceted or take other remarkable shapes. Their surface is sometimes nearly white and wax-like, but generally their color is variable. They are sometimes smooth, in other cases they are rough or uneven. The quantity of cholesterin in the stones varies from 642 to 981 p. m. (RITTER [1]). The cholesterin-stones sometimes contain variable amounts of lime-pigments, which may give them a very changeable appearance.

Cholesterin. The formula for this body, although not positively determined, is generally given as $C_{27}H_{46}O$ (OBERMÜLLER) or $C_{27}H_{44}O$ (MAUTHNER and SUIDA).

Because of the fact that from cholesterin, hydrocarbons which have been called *cholesteriline*, *cholesterone* and *cholesterilene*, can be prepared in different ways, it was believed that a certain analogy exists between the cholesterin and the terpenes. The color reactions as well as the recent investigations on the constitution of cholesterin indicate that this body belongs to the terpenes.

The constitution of cholesterin has not been completely determined, although we have the very laborious and thorough investigations of many workers of whom we especially mention MAUTHNER and SUIDA, WINDAUS, STEIN, DIELS and ABDERHALDEN.[2] From these investigations we conclude that cholesterin is a monoatomic, unsaturated, secondary alcohol whose hydroxyl group exists in a hydrogenized ring, between

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[1] Journ. de l'anat. et de la physiol. (Robin), 1872.

[2] The literature on cholesterin can be found in Windaus, Arch. d. Pharm., **246**, Hft. 2, and in Abderhalden's Bioch. Handlexikon, Bd., **3**, and also in Glikin, Bioch. Centralbl., 7, 372–377.

two methyl groups, and which also contains an isopropyl group. It is also generally admitted that cholesterin contains only one double bond, which occurs in a vinyl group, $CH:CH_2$, at the end. No constitutional formula for cholesterin can be given; still there is no doubt but that it is a complex terpene which stands in close relation to retene as well as to the cholic acids.

By the reduction of cholesterin by metallic sodium and amyl alcohol, DIELS and ABDERHALDEN as well as NEUBERG and RAUCHWERGER obtained a dihydrocholesterin, the *α-cholestanol*, $C_{27}H_{48}O$. On treating cholestenon, the ketone of cholesterin, DIELS and ABDERHALDEN obtained a second dihydrocholesterin, the *β-cholestanol*, which WILLSTÄTTER and E. W. MAYER obtained directly from cholesterin in ethereal solution by reduction with hydrogen and platinum-black. According to DIELS and LINN [1] β-cholestanol is obtained from cholestenon by the action of sodium and amyl alcohol, and α-cholestanol with sodium amylate. The relation of these bodies to each other is still not understood. These dihydrocholesterins have a physiological interest in regard to the question whether they are identical or not with koprosterin, which will be discussed below.

On heating cholesterin, when contaminated with iron, to 300–320°, according to DIELS and LINN,[2] it in part yields cholestenon and partly an isomeric cholesterin, the *β-cholesterin*. This last body can be retransformed into cholesterin by the saponification of the cholesteryl benzoate.

Cholesterin occurs in small amounts in nearly all animal fluids and juices. It occurs only rarely in the urine, and then in very small quantities. It is also found in the different tissues and organs, especially abundant in the brain and the nervous system; further, in the yolk of the egg, in semen, in wool-fat (together with isocholesterin), and in sebum. It also appears in the contents of the intestine, in excrements, and in the meconium. It especially occurs pathologically in gall-stones as well as in atheromatous cysts, in pus, in tuberculous masses, old transudates, cystic fluids, sputum, and tumors. It does not exist free in all cases; for example, it exists in part as fatty-acid esters in wool-fat, blood, lymph, brain, vernix caseosa and epidermis formations. Several kinds of cholesterin, called *phytosterines*, have been found in the vegetable kingdom.

Cholesterin which has been crystallized from warm alcohol on cooling, and also that which is present in old transudates, contains one molecule of water of crystallization, and melts at 148.5° C. when dried in a vacuum, and forms colorless, transparent plates whose sides and angles frequently appear broken, and whose acute angle is often 76° 30′ or 87° 30′. In large quantities it appears as a mass of white plates which shine like mother-of-pearl and have a greasy touch.

Cholesterin is insoluble in water, dilute acids, and alkalies. It is neither dissolved nor changed by boiling caustic alkali. It is easily

[1] Willstätter and Mayer, Ber d. d. chem. Gesellsch., 41; Diels and Linn, *ibid.*, 41.
[2] *Ibid.*, 41.

soluble in boiling alcohol, and crystallizes on cooling. It dissolves readily in ether, chloroform, and benzene, and also in the volatile or fatty oils. It is dissolved to a slight extent by alkali salts of the bile-acids, better in the presence of oleic soap (GERARD [1]). The solutions in ether and chloroform are levorotatory, $(\alpha)_D = -31.12°$ (2 per cent ethereal solution).

Among the many compounds of cholesterin the propionic ester $C_2H_5.CO.O.C_{27}H_{45}$ is of special interest because of the behavior of the fused compound on cooling, and it is used in the detection of cholesterin. For the detection of cholesterin use is made of its reaction with concentrated sulphuric acid, which gives colored products.

If a mixture of five parts sulphuric acid and one part water acts on cholesterin crystals, they show colored rings, first a bright carmine-red and then violet. This test is employed in the microscopic detection of cholesterin. Another test, and one very good for the microscopical detection of cholesterin, consists in treating the crystals first with the above dilute acid and then with some iodine solution. The crystals will be gradually colored violet, bluish-green, and a beautiful blue.

SALKOWSKI'S [2] *Reaction.* The cholesterin is dissolved in chloroform and then treated with an equal volume of concentrated sulphuric acid. The cholesterin solution becomes first bluish-red, then gradually more violet-red, while the sulphuric acid appears dark red with a greenish fluorescence. If the chloroform solution is poured into a porcelain dish it becomes violet, then green, and finally yellow.

LIEBERMANN-BURCHARD'S [3] *Reaction.* Dissolve the cholesterin in about 2 cc. chloroform and add first 10 drops of acetic anhydride and then concentrated sulphuric acid drop by drop. The color of the mixture will first be a beautiful red, then blue, and finally, if not too much cholesterin or sulphuric acid is present, a permanent green. In the presence of very little cholesterin the green color may appear immediately.

NEUBERG-RAUCHWERGER'S [4] *Reaction.* With rhamnose, or better still with δ-methylfurfurol and concentrated sulphuric acid, an alcoholic solution of cholesterin gives a pink ring, or after mixing the liquids and cooling, a pink solution. On proper dilution an absorption-band can be seen just beginning before *E* and whose other side coincides with *b*. This reaction is of interest because it is also given by bile-acids, some camphor derivatives, abietinic acid, and a hydride of retene.

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[1] Compt. rend. soc. biol., 58.

[2] Pflüger's Arch., 6.

[3] C. Liebermann, Ber. d. deutsch. chem. Gesellsch., 18; 1804, H. Burchard, Beiträge zur Kenntnis der Cholesterine, Rostock, 1899.

[4] Salkowski's Festschrift, 1904.

LIFSCHÜTZ'S[1] *Reaction.* Dissolve a few milligrams of cholesterin in 2–3 cc. glacial acetic acid, add a little benzoylsuperoxide thereto, and boil once or twice. On adding 4 drops concentrated sulphuric acid to the cooled solution and shaking, a pure green coloration is obtained, which changes immediately into blue or with violet-red as an intermediary color. An absorption-band is formed between *C* and *d*, and a broad band at *D*. In this reaction an oxidation of the cholesterin occurs, and LIFSCHÜTZ[2] therefore uses the glacial acetic acid-sulphuric acid reaction (color and spectrum) for the detection of oxidation products of cholesterin in blood and tissues.

Pure, dry cholesterin when fused in a test-tube over a low flame with two or three drops of propionic anhydride yields a mass which on cooling is first violet, then blue, green, orange, carmine-red, and finally copper-red. It is best to re-fuse the mass on a glass rod and then to observe the rod on cooling, holding it against a dark background (OBERMÜLLER).[3]

SCHIFF'S *Reaction.* If a little cholesterin is placed in a porcelain dish with the addition of a few drops of a mixture of 2 or 3 vols. of concentrated hydrochloric acid or sulphuric acid and 1 vol. of a rather dilute solution of ferric chloride, and carefully evaporated to dryness over a small flame, a reddish-violet residue is first obtained and then a bluish-violet.

If a small quantity of cholesterin is evaporated to dryness with a drop of concentrated nitric acid, one obtains a yellow spot which becomes deep orange-red with ammonia or caustic soda (not a characteristic reaction).

Cholesterin combines with saponin (WINDAUS, YAGI) and when a solution of cholesterin in boiling 95 per cent alcohol is treated with a warm 1 per cent solution of digitonin in 90 per cent alcohol, a precipitate of digitonin-cholesteride is obtained. If the amount of the washed and dried digitonin-cholesteride is multiplied by 0.25 the quantity of cholesterin is obtained (WINDAUS[4]). The cholesterin esters are not precipitated by digitonin.

Koprosterin is the name given by BONDZYNSKI to the cholesterin which was isolated by him from human feces, although it was prepared earlier by FLINT and designated as *stercorin*. It dissolves in cold, absolute alcohol and very readily in ether, chloroform, and benzene. It crystallizes in fine needles which melt at 95–96° C. (89–90° according to HAUSMANN), and is dextrorotatory $(\alpha)_D = +24°$. It gives the same color reactions as cholesterin, with the exception that it does not give a reaction with propionic anhydride. According to BONDZYNSKI and HUMNICKI it is a dihydrocholesterin, with the formula $C_{27}H_{48}O$, which is formed in the human intestine by the reduction of ordinary cholesterin. According to KUSUMOTO as well as DORÉE and GARDNER, koprosterin also occurs in the intestine of dogs. The koprosterin prepared by H. FISCHER from human feces seems to be identical with that prepared by BONDZYNSKI. It is remarkable that BOEHM[5]

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[1] Ber. d. d. chem. Gesellsch., **41**.

[2] Zeitschr. f. physiol. Chem., **50**, **53**, **58**, and Ber. d. d. chem. Gesellsch., **41**.

[3] Zeitschr. f. physiol. Chem., **15**.

[4] Windaus, Zeitschr. f. physiol. Chem., **65**; Yagi, Arch. f. exp. Path. u. Pharm., **64**.

[5] Bondzynski, Ber. d. deutsch. chem. Gesellsch., **29**; Bondzynski and Humnicki, Zeitschr. f. physiol. Chem., **22**; Flint, *ibid.*, **23**, and Amer. Journ. Med. Sciences, 1862; Müller, Zeitschr. f. physiol. Chem., **29**; Hausmann, Hofmeister's Beiträge, **6**; Kusumoto, Bioch. Zeitschr., **14**; Dorée and Gardner, Proc. Roy. Soc. London, **80**, Ser. B.; H. Fischer, Zeitschr. f. physiol. Chem., **73**; Boehm, Bioch. Zeitschr., **33**.

found a dihydrocholesterin in the contents in a part of the ileum which had been disconnected from the other part of the intestine for 14 years. This had the same optical rotation and the same melting-point, 142–143° C., as the dihydrocholesterin (β-cholestanol) prepared by DIELS and ABDERHALDEN, WILLSTÄTTER and MAYER.

Hippokoprosterin is another cholesterin richer in hydrogen, which BONDZYNSKI and HUMNICKI found in the feces of the horse. Its formula is $C_{27}H_{44}O$. According to DORÉE and GARDNER it is not an animal cleavage product, but a constituent of the grass used as fodder. It melts at 78.5–79.5° C.

Isocholesterin is a cholesterin, so called by SCHULZE,[1] with the formula $C_{26}H_{44}O$, which occurs in wool-fat, and is therefore found to a great extent in so-called lanolin. It gives the LIEBERMANN-BURCHARD reaction, but does not give SALKOWSKI's reaction. It melts at 138–138.5° C. The specific rotation in 7 per cent ethereal solution is $(\alpha)_D = +59.1°$.

Spongosterin, $C_{17}H_{28}O$ is the name given by HENZE[2] to a cholesterin isolated by him from a silicious sponge. It is very similar to cholesterin, but is not identical with it or with phytocholesterins. It gives the LIEBERMANN-BURCHARD reaction as well as SALKOWSKI's reaction, but the last test is not quite so beautiful a red. OBERMÜLLER's reaction is negative. Melting-point 123–124°.

Bombicesterin is the name given by MENOZZI and MORESCHI [3] to a cholesterin isolated by them from the chrysalis of the silkworm, which has a melting-point of 148° and a specific rotation of $(\alpha)_D = -34°$.

The cholesterin occurring in the intestine is derived in part from the food, in part from the bile and part, as shown from the contents of a ligatured portion of the intestine (see Chapter VIII), from the epithelium or the secretion of the intestinal mucosa. That a part of the cholesterin of the intestine disappears has been shown by KUSUMOTO, although it remains undecided whether this takes place by bacterial decomposition or by absorption. LEVITES [4] on the contrary, recovered the cholesterin introduced into dogs almost quantitatively. The behavior of cholesterin in metabolism is not well known; LIFSCHÜTZ believes that he has detected by his color-reaction the oxidation products of cholesterin in the blood and in bone-fat.

The cholesterins belong to the so-called lipoids, which have been mentioned in previous chapters (I and VI), and are of the greatest importance as constituents of the outer envelope of erythrocytes and the cells in general. Cholesterin is also of great interest because it inhibits or prevents the hæmolysis produced by certain bodies, and therefore acts as a certain protective power in the animal body. This action of the cholesterins in regard to inhibiting the hæmolytic action of saponin,

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[1] Ber. d. deutsch. chem. Gesellsch., **6**; Journal f. prakt. Chem. (N. F.), **25**; and Zeitschr. f. physiol. Chem., **14**, 522. See also E. Schulze and J. Barbieri, Journal f. prakt. Chem. (N. F.), **25**, 159. In regard to the formula for isocholesterin, see Darmstädter and Lifschütz, Ber. d. deutsch. chem. Gesellsch., **31**, and E. Schulze, *ibid.*, 1200.

[2] Zeitschr. f. physiol. Chem., **41** and **55**.

[3] Cited from Chem. Centralbl., 1908, 1377 and 1910, 872.

[4] Zeitschr. f. physiol. Chem., **57**.

as first discovered by RANSOM, is destroyed, as shown by HAUSMANN, by replacing the hydroxyl groups. These combinations between cholesterin and saponins have been studied by MADSEN and NOGUCHI and by WINDAUS.[1]

The so-called cholesterin-stones are best employed in the preparation of cholesterin. The powder is first boiled with water and then repeatedly boiled with alcohol. The cholesterin which on cooling separates from the warm filtered solution is boiled with a solution of caustic potash in alcohol so as to saponify any fat. After the evaporation of the alcohol the cholesterin is extracted from the residue with ether, by which the soaps are not dissolved; filter, evaporate the ether, and purify the cholesterin by recrystallization from alcohol-ether. The cholesterin may be extracted with fat from tissues and fluids by first extracting with ether and then proceeding as suggested by RITTER.[2] The essential points in his method consist in saponifying the fat with sodium alcoholate, removing the alcohol by evaporating to dryness with NaCl, and finally extracting the dried pulverized mass with ether. After evaporating the ether the residue is dissolved in as little alcohol as possible and the cholesterin precipitated by the addition of water. It is ordinarily easily detected in transudates and pathological formations by means of the microscope. In regard to the methods of preparation, detection and quantitative estimation of cholesterin we refer to the larger text-books.

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[1] Ransom, Deutsch. med. Wochenschr., 1901; Hausmann, Hofmeister's Beiträge, 6; Madsen and Noguchi, Kgl. Dansk. Vidensk. Selskabs. Forh., 1904; Windaus, Ber. d. d. chem. Gesellsch., 42.

[2] Zeitschr. f. physiol. Chem., 34. See also Corper, Journ. of biol. Chem., 11.

CHAPTER VIII.

DIGESTION.

THE purpose of digestion is to separate those constituents of the food which serve as nutriment for the body from those which are useless, and to separate each in such a form that it may be taken up by the blood from the alimentary canal and employed for various purposes in the organism. This demands not only mechanical, but also chemical, action. The first action, which is essentially dependent upon the physical properties of the food, consists in a tearing, cutting, crushing, or grinding of the food, while the second serves chiefly in converting the nutritive bodies into a soluble and easily absorbable form, or in splitting them into simpler compounds for use in the animal syntheses. The solution of the nutritive bodies may take place in certain cases by the aid of water alone, but in most cases a chemical metamorphosis or cleavage is necessary; this is effected by means of the acid or alkaline fluids secreted by the glands. The study of the processes of digestion from a chemical standpoint must therefore begin with the digestive fluids, their qualitative and quantitative composition, as well as their action on the nutriments and foods.

I. THE SALIVARY GLANDS AND THE SALIVA.

The **salivary glands** are partly *albuminous glands* (as the parotid in man and mammals, and the submaxillary in rabbits), partly *mucous glands* (as some of the small glands in the buccal cavity and the sublingual and submaxillary glands of many animals), and partly *mixed glands* (as the submaxillary gland in man). The alveoli of the albuminous glands contain cells which are rich in protein but which contain no mucin. The alveoli of the mucin-glands contain cells rich in mucin but poor in protein. Cells arranged in different ways, but rich in proteins, also occur in the submaxillary and sublingual glands. According to the analyses of MAGNUS-LEVY [1] the human salivary glands contain 274 p. m. solids, of which 114 p. m. was fat and 154 p. m. was protein. Among the solids we find *mucin, proteins, nucleoproteins, nuclein, enzymes* and their

[1] Bioch. Zeitschr., **24**.

zymogens, besides *extractive bodies*, *leucine*, *purine bases*, and *mineral substances*.

The occurrence of a mucinogen has not been proved. On the complete removal of all mucin E. HOLMGREN [1] found no mucinogen in the submaxillary gland of the ox, but a mucin-like gluconucleoproteid.

The **saliva** is a mixture of the secretion of the above-mentioned groups of glands; therefore it is proper that a study be made of each of the different secretions by itself and then of the mixed saliva.

The **submaxillary saliva** in man may be easily collected by introducing a canula through the papillary opening into Wharton's duct.

The submaxiliary saliva has not always the same composition or properties; this depends essentially, as shown by experiments on animals, upon the conditions under which the secretion takes place. That is to say, the secretion is partly dependent on the cerebral system, through the facial fibers in the chorda tympani, and partly on the sympathetic nervous system, through the fibers entering the vessels in the gland. In consequence of this dependence the two distinct varieties of submaxillary secretion are distinguished as *chorda-* and *sympathetic* saliva. A third kind of saliva, the so-called *paralytic saliva*, is secreted after poisoning with curare or after the severing of the glandular nerves.

The difference between chorda- and sympathetic saliva (in dogs) consists chiefly in their quantitative constitution; the less abundant sympathetic saliva is more viscous and richer in solids, especially in mucin, than the more abundant chorda-saliva. The specific gravity of the chorda-saliva of the dog is 1.0039–1.0056, and contains 12–14 p. m. solids (ECKHARD [2]). The sympathetic has a specific gravity of 1.0075–1.018, with 16–28 p. m. solids. The freezing-point of the chorda-saliva obtained from dogs on electric stimulation varies, according to NOLF,[3] between $\Delta = -0.193°$ and $-0.396°$, with a content of 3.3–6.5 p. m. salts and 4.1–11.5 p. m. organic substances. The osmotic pressure is on an average a little higher than one-half the osmotic pressure of the blood-serum. The spontaneously secreted submaxillary saliva is ordinarily somewhat diluted. On changing the osmotic pressure of the blood the osmotic pressure of the saliva, according to JAPPELLI,[4] changes in the same direction. According to DEMOOR, LOCKE'S solution with some dog serum is well suited by transfusion to keep the submaxillary gland of the dog in activity, while ox serum is unsuited.[5] The gases of the chorda-saliva

[1] Upsala Läkaref. Förh. (N. F.), **2**; also Maly's Jahresber., **27**.
[2] Cited from Kühne's Lehrb. d. physiol. Chem., **7**.
[3] See Maly's Jahresber., **31**, 494.
[4] Jappelli, *ibid.*, **48** and **51**.
[5] Arch. intern. de Physiol., **10** (1911).

have been investigated by PFLÜGER.[1] He found 0.5–0.8 per cent oxygen, 0.9–1 per cent nitrogen, and 64.73–85.13 per cent carbon dioxide—all results calculated at 0° C. and 760 mm. pressure. The greater part of the carbon dioxide was chemically combined.

The two kinds of submaxillary secretion just named have not thus far been separately studied in man. The secretion may be excited by an emotion, by mastication, and by irritating the mucous membrane of the mouth, especially with acid-tasting substances. The submaxillary saliva in man is ordinarily clear, rather thin, a little ropy, and froths easily. Its reaction is alkaline toward litmus. The specific gravity is 1.002–1.003, and it contains 3.6–4.5 p. m. solids.[2] As organic constituents are found mucin, traces of protein and diastatic enzyme, which latter is absent in several species of animals. The inorganic bodies are alkali chlorides, sodium and magnesium phosphates, and bicarbonates of the alkalies and calcium. Potassium sulphocyanide occurs in this saliva.

The Sublingual Saliva. The secretion of this saliva is also influenced by the cerebral and the sympathetic nervous system. The chorda-saliva, which is secreted only to a small extent, contains numerous salivary corpuscles, but is otherwise transparent and very ropy. Its reaction is alkaline, and it contains, according to HEIDENHAIN,[3] 27.5 p. m. solids (in dogs).

The sublingual secretion in man is clear, mucilaginous, more alkaline than the submaxillary saliva, and contains mucin, diastatic enzyme, and potassium sulphocyanide.

Buccal mucus can be obtained pure, from animals only, by the method suggested by BIDDER and SCHMIDT, which consists in tying the exit to all the large salivary glands and cutting off their secretion from the mouth. The quantity of liquid secreted under these circumstances (in dogs) was so very small that the investigators named were able to collect only 2 grams of buccal mucus in the course of one hour. It is a thick, ropy, sticky liquid containing mucin; it is rich in form-elements, above all in flat epithelium cells, mucous cells, and salivary corpuscles. The quantity of solids in the buccal mucus of the dog is, according to BIDDER and SCHMIDT,[4] 9.98 p. m.

Parotid Saliva. The secretion of this saliva is also partly dependent on the cerebral nervous system (n. glossopharyngeus) and partly on the sympathetic. The secretion may be excited by emotions and by irri-

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[1] Pflüger's Arch., 1.

[2] See Maly's "Chemie der Verdauungssäfte und der Verdauung," in Hermann's Handb., 5, part II, 18. This article contains also the pertinent literature.

[3] Studien. d. physiol. Instituts zu Breslau, Heft 4.

[4] Die Verdauungssäfte und der Stoffwechsel (Mitau and Leipzig, 1852), p. 5.

tation of the glandular nerves, either directly (in animals), or reflexly, by mechanical or chemical irritation of the mucous membrane of the mouth. Among the chemical irritants the acids take first place. Mastication also exercises a strong influence upon the secretion of parotid saliva, which is specially marked in certain herbivora.

Human parotid saliva may be readily collected by the introduction of a canula into STENSON'S duct. This saliva is thin, less alkaline than the submaxillary saliva (the first drops are sometimes neutral or acid), without special odor or taste. It contains a little protein but no mucin, which is to be expected from the construction of the gland. It also contains a diastatic enzyme, which, however, is absent in many animals. The quantity of solids varies between 5 and 16 p. m. The specific gravity is 1.003–1.012. Potassium sulphocyanide seems to be present, though it is not a constant constituent. KÜLZ [1] found a maximum of 1.46 per cent oxygen, 3.8 per cent nitrogen, and in all 66.7 per cent carbon dioxide in human parotid saliva. The quantity of firmly combined carbon dioxide was 62 per cent.

The quantity and composition of the saliva, from the mucin glands as well as from the albuminous glands, show differences in the various classes of animals but these cannot be entered into here. According to PAWLOW [2] and his pupils the quantity as well as the composition of the saliva of the various glands and the mixed saliva in dogs is to a great degree dependent upon the psychical stimulation, but also upon the kind of substances introduced into the mouth, and an adaptation of the glands for various mechanical and chemical irritants is found to occur.

POPIELSKI [3] disputes the existence of such an accommodation (in dogs) to the kind of food and to the kind of stimulation. In man an accommodation of the salivary glands, to the needs, has also been suggested but the statements are still not unanimous.[1] See also Chapter I (page 53).

The mixed buccal saliva in man is a colorless, faintly opalescent, slightly ropy, easily frothing liquid without special odor or taste. It is made turbid by epithelium cells, mucous and salivary corpuscles, and often by food residues. Like the submaxillary and parotid saliva, on exposure to the air it becomes covered with an incrustation consisting of calcium carbonate and a small quantity of an organic substance,

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[1] Zeitschr. f. Biologie, **23**.

[2] Arch. internat. de Physiol., **1**, 1904. See also Boos, Maly's Jahresber., **36**. 390, and Neilson and Terry, Amer. Journ. of Physiol., **15**, as well as the work of Mendel and Underhill, Journ. of biol. Chem., **3**.

[3] Popielski, Pflüger's Arch., **127**; Zebrowski, Pflüger's Arch., **110**; Neilson and Lewis, Journ. of biol. Chem., **4**, with Scheele, *ibid.*, **5**; Carlson and Chittenden, Amer. Journ. of Physiol., **26**.

or it gradually becomes cloudy. Its reaction is generally alkaline to litmus. The degree of alkalinity varies considerably not only in different individuals but also in the same individual during different parts of the day, so that it is difficult to state the average alkalinity. According to CHITTENDEN and ELY it corresponds to the alkalinity of 0.8 p. m. Na_2CO_3 solution, or to 0.2 p. m. solution according to COHN. According to FOA the actual alkalinity (OH-ion concentration) is always considerably less than that found by titration, and the reaction determined electrometrically is very nearly neutral. The reaction may also be acid, as found to be the case by STICKER some time after a meal, but this is not true, at least for all individuals. The specific gravity varies between 1.002 and 1.008, and the quantity of solids between 5 and 10 p. m. According to COHN,[1] $\Delta = -0.20°$ on an average, and the amount of NaCl is 1.6 p. m. The solids, irrespective of the form-constituents mentioned, consist of *protein*, *mucin*, *oxidases*,[2] two enzymes, *ptyalin* and *maltase*, as well as a dipeptid and a tripeptid splitting enzyme[3] and *mineral bodies*. It is also claimed that *urea* is a normal constituent of the saliva. The mineral bodies are alkali chlorides, bicarbonates of the alkalies and calcium, phosphates, and traces of sulphates, nitrites, ammonia, and sulphocyanides, which latter average about 0.1 p. m. (MUNK and others). Smaller quantities, 0.03–0.04 p. m., are found in the saliva of non-smokers (SCHNEIDER and KRÜGER), while from ordinary smokers the quantity of sulphocyanides may rise to 0.2 p. m. (FLECKSEDER[4]).

Sulphocyanides, which, although not constant, occur in the saliva of man and certain animals, may be easily detected by acidifying the saliva with hydrochloric acid and treating with a very dilute solution of ferric chloride. As control, especially in the presence of very small quantities, it is best to compare the test with another test-tube containing an equal amount of acidulated water and ferric chloride. Other methods have been suggested by GSCHEIDLEN, SOLERA, and GANASSINI. The quantitative estimation can be done according to MUNK'S[5] method.

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[1] Chittenden and Ely, Amer. Chem. Journ., 4, 1883; Chittenden and Richards, Amer. Journ. of Physiol., 1; Foa, Compt. rend. soc. biol., 58; Sticker, cited from Centralbl. f. Physiol., 8, 237; Cohn, Deutsch. med. Wochenschr., 1900.

[2] Bogdanow-Beresowski, cited from Biochem. Centralbl., 2, 653; Herlitzka, Maly's Jahresber., 40; Spanjer-Herford, Virchow's Arch., 205.

[3] Warfield, Johns Hopkins Hosp. Bull. 22 (1911); Koelker, Zeitschr. f. physiol. Chem., 76, (1911).

[4] Munk, Virchow's Arch., 69; Schneider, Amer. Journ. of Physiol., 5; Krüger, Zeitschr. f. Biologie, 37; Fleckseder, Centralbl. f. innere Med., 1905. In regard to the variation in the amount of various constituents in saliva see Fleckseder, l. c., and Tezner, Arch. internat. de Physiol., 2.

[5] Gscheidlen, Maly's Jahresber., 4; Solera, see *ibid.*, 7 and 8; Munk, Virchow's Arch., 69; Ganassini, Biochem. Centralbl., 2, p. 361.

Ptyalin, or salivary diastase, is the amylolytic enzyme of the saliva. This enzyme is found in human saliva,[1] but not in that of all animals, especially not in the typical carnivora. It occurs not only in adults, but also in new-born infants. In opposition to ZWEIFEL'S views, BERGER[2] claims that it is present not only in the parotid gland of children, but also in the mucin glands.

According to H. GOLDSCHMIDT[3] the saliva (parotid saliva) of the horse does not contain ptyalin, but a zymogen of the same, while in other animals and man the ptyalin is formed from the zymogen during secretion. In horses the zymogen is transformed into ptyalin during mastication, and bacteria seem to give the impulse to this change. During precipitation with alcohol the zymogen is changed into ptyalin.

Ptyalin has not been isolated in a pure form up to the present time. It can be obtained purest by COHNHEIM'S[4] method, which consists in carrying the enzyme down mechanically with a calcium-phosphate precipitate, and washing the precipitate with water, which dissolves the ptyalin, and from which it can be obtained by precipitating with alcohol. For the study or demonstration of the action of ptyalin one employs a watery or glycerin extract of the salivary glands, or simply the saliva itself.

Ptyalin, like other enzymes, is characterized by its action. This consists in converting starch into dextrins and sugar. Our knowledge as to the process going on here is just as uncertain as our knowledge on the formation of sugar from starch (see page 229). The nature of the sugar thus produced is known with certainty. For a long time it was considered that glucose was the sugar formed from starch and glycogen, but SEEGEN and O. NASSE have shown that this is not true. MUCULUS and v. MERING have shown that the sugar formed by the action of saliva, amylopsin, and diastase upon starch and glycogen is for the most part maltose. This has been substantiated by BROWN and HERON. E. KÜLZ and J. VOGEL[5] have also demonstrated that in the saccharification of starch and glycogen, isomaltose, maltose, and some glucose are formed, the varying quantities depending upon the amount of ferment and the length of its action. The formation of

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[1] In regard to the variation in the quantity of ptyalin in human saliva see Hofbauer, Centralbl. f. Physiol., **10**, and Chittenden and Richards, Amer. Journ. of Physiol., **1**; Schüle, Maly's Jahresber., **29**; Tezner, l. c.

[2] Zweifel, Untersuchungen über den Verdauungsapparat der Neugeborenen (Berlin, 1874); Berger, see Maly's Jahresber., **30**, 399.

[3] Zeitschr. f. physiol. Chem., **10**.

[4] Virchow's Arch., **28**.

[5] Seegen, Centralbl. f. d. med. Wissensch., 1876, and Pflüger's Arch., **19**; Nasse, *ibid.*, **14**; Musculus and v. Mering, Zeitschr. f. physiol. Chem., **2**; Brown and Heron, Liebig's Annal., **199** and **204**; Külz and Vogel, Zeitschr. f. Biologie, **31**.

glucose is claimed by TEBB, RÖHMANN, and HAMBURGER[1] to be only a product of the inversion of the maltose by the maltase.

The action of ptyalin in various *reactions* has been the subject of numerous investigations.[2] Natural alkaline saliva is very active, but it is not so active as when made neutral. It may be still more active under certain circumstances in faintly acid reaction, and according to CHITTENDEN and SMITH it acts better when enough hydrochloric acid is added to saturate the proteins present than when only neutralized. When the acid-combined protein exceeds a certain amount, then the diastatic action is diminished. The addition of alkali to the saliva decreases its diastatic action; on neutralizing the alkali with acid or carbon dioxide the retarding or preventive action of the alkali is arrested. According to SCHIERBECK, carbon dioxide has an accelerating action in neutral liquids, while EBSTEIN claims that it has, as a rule, a retarding action. Organic as well as inorganic acids, when added in sufficient quantity, may stop the diastatic action entirely. The degree of acidity necessary in this case is not always the same for a certain acid, but is dependent upon the quantity of ferment. The same degree of acidity in the presence of large amounts of ferment has a weaker action than in the presence of smaller quantities. Hydrochloric acid is of special physiological interest in this regard, for it prevents the formation of sugar even in very small amounts (0.03 p. m.). Hydrochloric acid has not only the property of preventing the formation of sugar, but, as shown by LANGLEY, NYLÉN, and others, may entirely destroy the enzyme. This is important in regard to the physiological significance of the saliva.

Foreign substances, such as metallic salts,[3] have different effects. Certain salts, even in small quantities, completely arrest the action; for example, $HgCl_2$ accomplishes this result completely in the presence of only 0.05 p. m. Others have an accelerating action, and this seems to apply to the salts of the saliva. According to GUYENOT the saliva has a weaker action the more it is freed from salts by dialysis. On the

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[1] Tebb, Journ. of Physiol., **15**; Röhmann, Ber. d. deutsch. chem. Gesellsch., **27**; Hamburger, Pflüger's Arch., **60**.

[2] See Hammarsten, Maly's Jahresber., 1; Chittenden and Griswold, Amer. Chem. Journ., **3**; Langley, Journal of Physiol., **3**; Nylén, Maly's Jahresber., **12**, 241; Chittenden and Ely, Amer. Chem. Journ., **4**; Langley and Eves, Journal of Physiol., **4**; Chittenden and Smith, Yale College Studies, **1**, 1885, 1; Schlesinger, Virchow's Arch., **125**; Schierbeck, Skand. Arch. f. Physiol., **3**; Ebstein and C. Schulze, Virchow's Arch., **134**; Kübel, Pflüger's Arch., **56**.

[3] See O. Nasse, Pflüger's Arch., **11**, and Chittenden and Painter, Yale College Studies, **1**, 1885, 52; Kübel, Pflüger's Arch., **76**; Patten and Stiles, Amer. Journ. of Physiol., **17**.

addition of salts the dialyzed saliva becomes active again, especially on the addition of calcium or potassium chloride (see also page 71). ROGER[1] believes that the presence of phosphates is a necessity for the action of saliva. The amount of salts added is of special importance for the action of the saliva, and one salt, which in small quantities has an accelerating action, may in large quantities have a retarding action. The presence of peptone has an accelerating action on the sugar formation (CHITTENDEN and SMITH and others).

To show the action of saliva or ptyalin on starch the three ordinary tests for glucose may be used, namely, MOORE'S or TROMMER'S test or the *bismuth test* (see Chapter III). It is also necessary, as a control, to first test the starch-paste and the saliva for the presence of glucose. The steps in the transformation of starch into amidulin, erythrodextrin, and achroödextrin may be shown by testing with iodine.

Maltase occurs in saliva to only a slight extent. It converts maltose into glucose. According to STICKER,[2] saliva also has the power of splitting sulphureted hydrogen from the sulphur oils of radishes, onions, and certain other vegetables.

The *quantitative composition* of the mixed saliva must vary considerably, not only because of individual differences, but also because under varying conditions there may be an unequal division of the secretion from the different glands. We give herewith a few analyses of human saliva as examples of its composition. The results are in parts per 1000.

	BERZELIUS.	JACUBOWITSCH.	FRERICHS.	TIEDEMANN and GMELIN.	HEINTZ.	LEHMANN.	HAMMERBACHER.[3]
Water	992.9	995.16	994.1	988.3	994.7	.....	994.2
Solids	7.1	4.84	5.9	11.7	5.3	3.5–8.4 in filtered saliva.	5.8
Mucus and epithelium	1.4	1.62	2.13	.....	.....	.....	2.2
Soluble organic substances. (Ptyalin of early investigators.)	3.8	1.34	1.42	.....	3.27	.....	1.4
Sulphocyanides	.....	0.06	0.10	.....	.....	0.064 to 0.090	0.04
Salts	1.9	1.82	2.19	.....	1.30	.....	2.2

[1] Guyenot, Compt. rend. soc. biol., **63**; Roger, *ibid.*, **65**; see also Bang, Bioch. Zeitschr., **32** (1911).

[2] Münch. med. Wochenschr., **43**.

[3] Zeitschr. f. physiol. Chem., **5**. The other analyses are cited from Maly, Chemie der Verdauungssäfte, Hermann's Handbuch d. Physiol., **5**, Part II, 14.

HAMMERBACHER found in 1000 parts of the ash from human saliva: potash 457.2, soda 95.9, iron oxide 50.11, magnesia 1.55, sulphuric anhydride (SO_3) 63.8, phosphoric anhydride (P_2O_5) 188.48, and chlorine 183.52.

The quantity of saliva secreted during twenty-four hours cannot be exactly determined, but has been calculated by BIDDER and SCHMIDT to be 1400–1500 grams. The most abundant secretion occurs during meal-times. According to the calculations and determinations of TUCZEK,[1] in man 1 gram of gland yields 13 grams of secretion in the course of one hour during mastication. These figures correspond fairly well with those representing the average secretion from 1 gram of gland in animals, namely, 14.2 grams in the horse and 8 grams in oxen. The quantity of secretion per hour may be 8 to 14 times greater than the entire mass of glands, and there is probably no gland in the entire body, so far as is known at present—the kidneys not excepted—whose ability of secretion under physiological conditions equals that of the salivary glands. But as the secretion of saliva is so very variable under different conditions no positive results can be given as to the extent. A remarkably abundant secretion of saliva is induced by pilocarpine, while atropine, on the contrary, inhibits it.

That the secretion of saliva, even if we do not consider such substances as ptyalin, mucin, and the like, is not a process of filtration, follows for many reasons, especially the following: The salivary glands have a specific property of eliminating certain substances, such as potassium salts (SALKOWSKI[2]), iodine, and bromine compounds, but not others, for example, iron compounds and glucose. It is also noticeable that the saliva is richer in solids when it is eliminated quickly by gradually increased stimulation, and in larger quantities than when the secretion is slower and less abundant (HEIDENHAIN). The amount of salts increases also to a certain degree by an increasing rapidity of elimination (HEIDENHAIN, WERTHER, LANGLEY and FLETCHER, NOVI[3]).

Like the secretion processes in general, the secretion of saliva is closely connected with the processes in the cells. The chemical processes going on in these cells during secretion are still unknown.

The Physiological Importance of the Saliva.—The quantity of water in the saliva renders possible the action of certain bodies on the organs of taste, and it also serves as a solvent for a part of the nutritive substances. The importance of the saliva in mastication is especially marked in herbivora, and there is no question as to its importance in

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[1] Bidder and Schmidt, l. c., 13; Tuczek, Zeitschr. f. Biologie, 12.

[2] Virchow's Arch., 53.

[3] Heidenhain, Pflüger's Arch., 17; Werther, *ibid.*, 38; Langley and Fletcher, Proc. Roy. Soc., 45, and especially Phil. Trans. Roy. Soc. London, 180; Novi, Arch. f. (Anat. u.) Physiol., 1888.

facilitating the act of swallowing. The saliva containing mucin is especially important in this regard, and PAWLOW's school has shown that the secretion also regulates itself in this regard. The saliva is also of importance, as it serves in washing out the mouth and thereby acts as a protection against destructive substances or bodies foreign to the mouth. The power of converting starch into sugar is not inherent in the saliva of all animals, and even when it possesses this property the intensity varies in different animals. In man, whose saliva forms sugar rapidly, a production of sugar from (boiled) starch undoubtedly takes place in the mouth, but how far this action proceeds after the morsel has entered the stomach depends upon the rapidity with which the acid gastric juice mixes with the swallowed food, and also upon the relative amounts of the gastric juice and food in the stomach. The large quantity of water which is swallowed with the saliva must be absorbed and pass into the blood, and it must in this way go through an intermediate circulation in the organism. Thus the organism possesses in the saliva an active medium by which a constant stream, conveying the dissolved and finely divided bodies, passes into the blood from the intestinal canal during digestion. The relation of the saliva or the salivary glands to the secretion of gastric juice will be mentioned in the next section.

Salivary Concrements. The so-called tartar is yellow, gray, yellowish-gray, brown or black, and has a stratified structure. It may contain more than 200 p. m. organic substances, which consist of mucin, epithelium, and LEPTOTHRIX-CHAINS. The chief part of the inorganic constituents consists of calcium carbonate and phosphate. The salivary calculi may vary in size from that of a small grain to that of a pea or still larger (a salivary calculus has been found weighing 18.6 grams), and they contain variable quantities of organic substances (50–380 p. m.), which remain on extracting the calculus with hydrochloric acid. The chief inorganic constituent is calcium carbonate.

II. THE GLANDS OF THE MUCOUS MEMBRANE OF THE STOMACH, AND THE GASTRIC JUICE.

The glands of the mucous coat of the stomach have long been divided into two distinct classes. Those which occur in the greatest abundance and which have the greatest size in the fundus are called *fundus*, rennin or pepsin glands, and the others, which occur only in the neighborhood of the pylorus, have received the name of *pyloric glands*, sometimes also, though incorrectly, called *mucous glands*. The division of these two forms of glands in the mucous membrane of the stomach is essentially different in various animals. The mucous coating of the stomach is covered throughout with a layer of columnar epithelium, which is generally considered as consisting of goblet cells that produce mucus by a metamorphosis of the protoplasm.

The **fundus glands** contain two kinds of cells: ADELOMORPHIC or chief cells, and DELOMORPHIC or COVER cells, the latter formerly called RENNIN or pepsin cells. Both kinds consist of protoplasm rich in proteins; but their relation to coloring-matters seems to show that the protein substances of both are not identical. The nucleus must consist principally of nuclein. Besides the above-mentioned constituents, the fundus glands contain as more specific constituents several *enzymes* or their *zymogens*, besides a little fat and cholesterin.

The **pyloric glands** contain cells which are generally considered as related to the above-mentioned chief cells of the fundus glands. As these glands were formerly thought to contain a larger quantity of mucin, they were also called mucous glands. According to HEIDENHAIN, independent of the columnar epithelium of the excretory ducts, they take no part worthy of mention in the formation of mucus, which according to his views is effected by the epithelium covering the mucous membrane. The pyloric glands also seem to contain *zymogens*. Alkali chlorides, alkali phosphates, and calcium phosphates are found in the mucous coating of the stomach.

The Gastric Juice. The observations of HELM and BEAUMONT on persons with gastric fistula led to the suggestion that gastric fistulas be made on animals, and this operation was first performed by BASSOW [1] in 1842 on a dog. VERNEUIL performed the same on a man in 1876 with successful results. PAWLOW [2] has recently improved the surgery of gastric fistula and has added much to the study of gastric secretion.

As most investigations upon gastric digestion, and also upon digestion as a whole, are based on observations upon dogs and then upon man, and for this reason, when not otherwise stated, in this chapter on the study of digestion we give the conditions in dogs and man.

The secretion of gastric juice is not continuous, at least in man and in the mammals experimented upon. It only occurs under psychic influence, and also by stimulation of the mucous membrane of the stomach or the intestine. The most exhaustive researches on the secretion of gastric juice (in dogs) have been made by PAWLOW and his pupils.

In order to obtain gastric juice free from saliva and food residues, they arranged besides a gastric fistula also an œsophageal fistula from which the swallowed food could be withdrawn with the saliva without entering the stomach, and in this manner an apparent or sham feeding was possible. In this way it was possible to

[1] Helm, Zwei Krankengeschichten, Wien, 1803, cited from Hermann's Handbuch, **5**, part II, 39; Beaumont, "The Physiology of Digestion," 1833; Bassow, Bull. de la soc. des natur. de Moscou, **16**, cited from Maly in Hermann's Handbuch, **5**, 38; Verneuil, see Ch. Richet, "Du suc gastrique chez l'homme," etc. (Paris, 1878), 158.

[2] Pawlow, The Work of the Digestive Glands, (translated by Thompson, Philadelphia, 1910), where the works of his pupils are also mentioned. See also Ergebnisse der Physiologie, 1, Abt. 1.

study the influence of psychical moments on one side and the direct action of food on the mucous membrane on the other. After a method suggested by HEIDENHAIN and later improved by PAWLOW and CHIGIN, they have succeeded in preparing a blind sac by partial dissection of the fundus part of the stomach, and the secretion processes could be studied in this sac while the digestion in the other parts of the stomach was going on. In this way they were able to study the action of different foods on the secretion.

The most essential results of the investigations of PAWLOW and his pupils are as follows: Mechanical stimulation of the mucosa does not produce any secretion. Mechanical irritation of the mucous membrane of the mouth causes no reflex excitation of the secretory nerves of the stomach. There are two moments which cause a secretion, namely, the psychical moment—the passionate desire for food and the sensation of satisfaction and pleasure on partaking it—and the chemical moment, the action of certain chemical substances on the mucous membrane of the stomach. The first moment is the most important. The secretion occurring under its influence by the vagus fibers appears earlier than that produced by chemical irritants, but only after an interval of at least 4½ minutes. This secretion is more abundant but less continuous than the "chemical." It yields a more acid and active juice than the latter. As chemical excitants which cause a secretion reflexively through the stomach mucosa we include water (slight action) and certain unknown extractive substances contained in meat and meat extracts, in impure peptone, and also, it seems, in milk. According to HERZEN and RADZIKOWSKI [1] and others, alcohol is also a strong agent in producing a flow of juice. The claims in regard to the action of sodium chloride and alkali carbonates are somewhat disputed. That the alkali carbonates retard or inhibit secretion is the opinion of many, but from more recent determinations [2] it would seem as if the concentration of the carbonate as well as of sodium chloride exercises a certain influence, so that a weaker concentration is indifferent or retarding, while somewhat stronger concentration has an accelerating action upon secretion, though investigators are not agreed as to results. Bitter substances partaken of in small amounts a certain time before a meal increase the secretion, while larger amounts have a retarding action (BORISSOW, STRASHESKO [3]). Fats have a retarding action on the appearance of secretion and diminish the quantity of juice secreted as well as the amount of enzyme. The substances, such as egg-albumin, which do not act as chemical stimulants,

[1] Pflüger's Arch., 84, 513.

[2] See Rozenblatt, Bioch. Zeitschr, 4; Mayeda, *ibid.*, 2; Pimenow, Bioch, Centralbl., 6; Lönnquist, Maly's Jahresb., 36.

[3] Borissow, Arch. f. exp. Path. u. Pharm., 51; Strashesko, see Biochem. Centralbl., 4, 148.

may be digested by the "psychical" secretion, and then perhaps cause a chemical secretion by their decomposition products.

The secretion in the stomach may also be influenced by the small intestine, and in this way, as shown by the investigations of PAWLOW and his pupils, the fats have a retarding action upon the secretion of juice and upon digestion by acting reflexly upon the duodenal mucosa. In dogs on feeding fat (oil) with food containing starch, the secretion of gastric juice remains reduced during the entire period of feeding, and fat in connection with protein food has a similar action, with the exception that in this case the retarding action is observed only in the first hours of digestion. According to PIONTKOWSKI [1] the oil-soaps differ from the neutral fats by having a strong action on the flow of juice, and this is the reason why about five to six hours after a meal with fat the secretion of juice is stopped, as just at this time the soaps are being formed. According to FROUIN the food in the intestine produces a secretion of gastric juice which continues after the action of the psychic moment has ceased. LECONTE [2] arrived at similar results, and he ascribes a less subordinate importance to the chemical secretion as compared with the psychic secretion, than PAWLOW does.

The behavior of the different parts of the stomach in secretion is also of interest. The work of PAWLOW and his pupils GROSS and KRSHYSCHKOWSKY, has shown that meat and its extractives as well as the digestion products and milk especially act upon the pyloric part, although not entirely, while they are inactive upon the fundus. Alcohol also acts upon the fundus part. POPIELSKI [3] found that meat extracts had an exciting action upon the secretion of gastric juice, even when introduced subcutaneously. In close relation to what has been said above stands the observation of EDKINS that the pylorus part of the stomach contains a substance, a *prosecretin*, which by acids and certain other substances is transformed into a *secretin*, which when introduced into the blood circulation causes a secretion of gastric juice. HEMMETER, claims that a secretin for the secretion of gastric juice is also produced in the salivary glands. The extirpation of all the salivary glands in dogs causes a marked diminution in the secretion of gastric juice, while the intravenous or peritoneal injection of an extract of the salivary glands of dogs produces a secretion of gastric juice. EMSMANN [4] has also obtained bodies having a similar action, from the mucosa of the

[1] See Biochem. Centralbl., **8**, 660.

[2] Frouin, Compt. rend. soc. biol., **58**; Leconte, La Cellule, **17**.

[3] Gross, Bioch. Centralbl., **5**, 669; Krshyschkowsky, Maly's Jahresb., **36**, 403; Popielski, *ibid.*, **39**.

[4] Edkins, Journ. of Physiol., **34**; Hemmeter, Bioch. Zeitschr., **11**; Emsmann, Intern. Beitr. zu. Path. u. Ther. d. Ernahrungs störungen **3** (1911).

duodenum, jejunum, and ileum as well as from the liver and pancreas by hydrochloric acid.

We know very little, positively, in regard to the gastric secretion in man. According to the earlier authorities the irritants may be mechanical, thermic, and chemical. Among the chemical excitants we include alcohol and ether, which in too great a concentration bring about no physiological secretion, but rather the transudation of a neutral or faintly alkaline fluid. Certain acids, such as carbonic acid, neutral salts, meat extracts, spices, and other bodies also belong to this group. The reports on this subject are unfortunately very uncertain and contradictory.

The question as to how far the observations made by PAWLOW and his school can be applied to man is of special interest. Many observations on this question have been collected [1] and they compare favorably with the observations made upon dogs. Thus in man a psychic secretion of gastric juice can be brought about, and it has also been observed that it can be stopped by emotions. As in dogs, so also in man, after sham feeding, a secretion takes place after a pause, the duration of which varies in different cases. In some cases, as in dogs after meat feeding, the pause was about five minutes. The chewing of indifferent bodies did not affect the glands, while bodies acting upon the organs of smell and taste had an exciting action. UMBER observed besides this, that after the introduction of a nutritive enema into the rectum, a secretion of gastric juice was produced by reflex action.

From these observations of HORNBORG and UMBER, as well as from some earlier observations of SCHÜLE, TROLLER, RIEGEL, and SCHEUER,[2] we conclude that in man the psychic secretion is much less than that produced by the introduction of food or bodies having a pleasant taste. That the preparation of the food in the mouth has an essential influence upon the secretion is proved without doubt, but we do not agree as to how this action takes place. Certain experimenters consider the secreted and swallowed saliva as the most essential factor in this action, while others believe that the act of chewing, and still others that the chemical action and the sense of taste, are the most important.

In regard to the action of saliva, HEMMETER finds that after the extirpation of the salivary glands, the introduction into the stomach of chewed food soaked with dog-saliva, has no special action upon the

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[1] Hornborg, Maly's Jahresb., **33**, 547; Umber, Berl. klin. Wochenschr., 1905; Cade and Latarjet, Compt. rend. soc. biol., **57**; Kaznelson, Pflüger's Arch., **118**; Bogen, *ibid.*, **117**; Bickel, Deutsch. med. Wochenschr., **32**, and Maly's Jahresb., **36**, 411. See also Maly's Jahresb. **39**, **40**, and Bioch, Centralbl. **12**.

[2] The literature may be found in Umber's work, l. c.

secretion of juice. On the other hand FROUIN [1] observed that the introduction of saliva into the large stomach of dogs, acts favorably upon the secretion in the small stomach (see page 462), and the acidity as well as the digestive activity of the juice is increased. This action does not depend, according to FROUIN, upon the alkali of the saliva.

The Qualitative and Quantitative Composition of the Gastric Juice. The human gastric juice, which can seldom be obtained pure and free from residues of the food or from mucus and saliva, is a clear, or only very faintly cloudy, and nearly colorless fluid of an insipid, acid taste and strong acid reaction. It contains, as form-elements, *glandular cells* or their *nuclei*, and more or less changed *columnar epithelium*.

The acid reaction of the gastric juice depends on the presence of free acid, which, as has been learned from the investigations of C. SCHMIDT, RICHET, and others, consists, when the gastric juice is pure and free from particles of food, chiefly or in large part of *hydrochloric acid*. CONTEJEAN [2] regularly found traces of lactic acid in the pure gastric juice of fasting dogs. After partaking of food, especially after a meal rich in carbohydrates, lactic acid occurs abundantly, and sometimes acetic and butyric acids. In new-born dogs the acid of the stomach is lactic acid, according to GMELIN.[3] The quantity of free hydrochloric acid in the gastric juice is, according to PAWLOW and his pupils, in dogs 5–6 p. m., and in cats an average of 5.20 p. m. HCl. In man the results obtained are variable but regularly much lower. Since it has been possible to obtain pure human gastric juice for investigation it has been found (UMBER, HORNBORG, BICKEL, SOMMERFELD [4]) that the amount of hydrochloric acid is about 4–5 p. m. There is hardly any doubt that at least a part of the hydrochloric acid of the gastric juice does not exist free in the ordinary sense, but combined with organic substances. The results obtained in testing for the acidity of gastric juice by physical methods are almost identical with those obtained by titration (P. FRÄNCKEL [5]).

The specific gravity of gastric juice is low, 1.001–1.010. It is correspondingly poor in solids. Earlier analyses of gastric juice from man, the dog, and the sheep were made by C. SCHMIDT.[6] As these analyses

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[1] Compt. rend. soc. biol., **62**.

[2] Bidder and Schmidt, Die Verdauungssäfte, etc., **44**; Richet, l. c.; Contejean, Contributions a l'étude de la physiol. de l'estomac, Thèses, Paris, 1892.

[3] Pflüger's Arch., **90** and **103**.

[4] See Richet, l. c.; Contejean, l. c.; Verhaegen, "La Cellule," 1896 and 1897; Sommerfeld, Bioch, Zeitschr, **9**, and also footnote 1, page 464, and the literature on the estimation of hydrochloric acid in the gastric juice contents (p. 489); see also Cohnheim and Dreyfus, Zeitschr. f. physiol. Chem. **58** (1908).

[5] Zeitschr, f. exp. Path. u. Therap., **1**.

[6] l. c.

refer only to impure gastric juice they are of little value. ROSEMANN,[1] who has investigated the gastric juice secreted by a dog after sham feeding, found an average of 4.22 p. m. solids, among which 1.32 p. m. were mineral bodies and about 2.90 p. m. organic substance. The amount of nitrogen in one case was 0.36 p. m., in another 0.54 p. m. and the quantity of HCl was about 5.6 p. m. The ash consisted chiefly of potassium chloride, namely 980–990 p. m. of the inorganic part. NENCKI and SIEBER[2] found 3.06 p. m. solids in the pure gastric juice of a dog. NENCKI[3] found 5 milligrams sulphocyanic acid per liter of gastric juice of a dog.

In the ash of human gastric juice after sham-feeding ALBU[4] found 356.2 p. m. K_2O; 226.5 p. m. Na_2O, and 497.3 p. m. Cl. The amount of salts insoluble in water was 23.9 p. m. In hyperacidity he found almost the same composition.

Besides the free hydrochloric acid, *pepsin*, *rennin*, and a *lipase* are the other physiologically important constituents of gastric juice.

Pepsin. This enzyme is found, with the exception of certain fishes, in all vertebrates thus far investigated.

Pepsin occurs in adults and in new-born infants. This condition is different in new-born animals. While in a few herbivora, such as the rabbit, pepsin occurs in the mucous coat before birth, this enzyme is entirely absent at the birth of those carnivora which have thus far been examined, such as the dog and cat.

In various invertebrates enzymes have also been found which have a proteolytic action in acid solutions. It has been shown that these enzymes, nevertheless, are not in all animals identical with ordinary pepsin. According to KLUG and WRÓBLEWSKI[5] the pepsins found in man and various higher animals are somewhat different, an observation which according to the experience of HAMMARSTEN is very probable. Enzymes also occur in various plants and animal organs, although not identical with pepsin, but which act in acid reaction. The enzyme obtained from the Nepenthes, which dissolves proteins only in acid reaction, stands very close to pepsin. An enzyme more closely related to trypsin or erepsin (see sections III and IV) is, on the contrary, GLAESSNER'S *pseudopepsin*, which according to him is the only peptic enzyme in the pyloric end. Pseudopepsin, whose existence is disputed by KLUG, while others (REACH, PEKELHARING) affirm its occurrence in

[1] Pflüger's Arch., 118.
[2] Zeitschr. f. physiol. Chem., 32.
[3] Ber. d. d. Chem. Gesellsch., 28.
[4] Zeitschr. f. Path. u. Therap., 5.
[5] Klug. Pflüger's Arch. 60; Wróblewski, Zeitschr. f. physiol. Chem., 21.

the mucous membrane, cannot, according to HAMMARSTEN, either be the only or the most prominent peptic enzyme of the pyloric part. According to GLAESSNER, it also acts in neutral and alkaline reaction and yields tryptophane among other cleavage products. According to BERGMANN [1] it is identical with erepsin (see below). Among the enzymes of the mucosa of the stomach belongs the so-called *antipepsin* discovered by WEINLAND,[2] which has a retarding action upon pepsin digestion and, as some claim, prevents the self-digestion of the mucous membrane.

Pepsin is as difficult to isolate in a pure condition as are other enzymes. The pepsin prepared by BRÜCKE and SUNDBERG gave negative results with most reagents for proteins, and showed nevertheless a powerful action, which seems to indicate that it was very pure. SCHOUMOW-SIMANOWSKI, NENCKI and SIEBER, have designated as the true enzyme the substance containing chlorine, which they obtain by strongly cooling the gastric juice. That this precipitate is not a chemical individual, and hence cannot be pepsin, follows from the investigations of PEKELHARING. While pepsin, according to NENCKI and SIEBER, was rich in phosphorus and contained nucleoprotein, PEKELHARING'S pepsin was free from phosphorus and yielded no nucleoprotein. FRIEDENTHAL and MIYAMOTA [3] have also shown that the pepsin is still active after the splitting off of the nuclein complex (and also the protein). As pepsin is readily precipitated with the proteins and combines therewith, it is difficult to decide whether pepsin is a protein substance or not, and the question as to its nature is still undecided, just as is the case with other enzymes. As ordinarily known, pepsin, at least in an impure form, is soluble in water and glycerin. It is precipitated by alcohol, but is only slowly destroyed thereby. In aqueous solution its action is quickly destroyed on heating to boiling. According to BIERNACKI [4] pepsin in neutral solutions is destroyed by heating to 55° C. In the dry state it can be heated to over 100° C. without losing its activity. In the presence of 2 p. m. HCl a temperature of 55° C. is not injurious, and the compound with acid is more resistant than the free pepsin (GROBER [5]). Pepsin in acid solution is destroyed by heating to 65° C. for five minutes.

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[1] Glaessner, Hofmeister's Beiträge, 1; Klug, Pflüger's Arch., 92; Reach, Hofmeister's Beiträge, 4; Pekelharing, Arch. des scienc., biolog., St. Pétersbourg 11; Pawlow-Festband, 1904; Bergmann, Skand. Arch. f. Physiol., 18.

[2] Zeitschr. f. Biologie, 44.

[3] Brücke, Wien. Sitzungsber,. 43; Sundberg, Zeitschr. f. physiol. Chem., 9; Schoumow-Simanowski, Arch. f. exp. Path. u. Pharm., 33; Pekelharing, Zeitschr. f. physiol. Chem., 22 and 35; Nencki and Sieber, *ibid.*, 32; Friedenthal and Miyamota, Centrabl. f. Physiol., 15, 785.

[4] Zeithschr. f. Biologie, 28.

[5] Arch. f. exp. Path. u. Pharm., 51.

On adding peptone or certain salts the pepsin may be heated to 70° C. for the same time without destruction.

The behavior of pepsin on heating its acid solution is influenced not only by the degree of acidity, but by the duration of heating and also by the amount of other bodies in the solution. If an acid (0.2 per cent HCl) infusion of the calf's stomach be warmed for several days to about 40 or 45° C., a part of the pepsin is destroyed, but we obtain in this manner an infusion which still dissolves proteins but has no rennin action (HAMMARSTEN [1]). The pepsin from different animals acts differently in this regard and the pepsin of the pike stomach is very quickly destroyed at 37–40° C.

Pepsin is extraordinarily sensitive to the action of alkalies, not only caustic, and carbonated, but also against the hydroxides of the alkaline earths. It is easily made inactive by these substances. If the action of the alkali is not too strong then, as shown by PAWLOW and TICHOMIROW,[2] the enzyme can in part be reactivated by the addition of acid if the greater part (about four-fifths), of the alkalinity be neutralized by the addition of acid and then after some hours more acid be added. If the entire quantity of acid be added at one time the reactivation does not take place.

The only property which is characteristic of pepsin is that it dissolves protein bodies in acid but not in neutral or alkaline solutions, with the formation of proteoses, peptones, and other products.

The methods for the preparation of relatively pure pepsin depend, as a rule, upon its property of being thrown down with finely divided precipitates of other bodies, such as calcium phosphate or cholesterin. The rather complicated methods of BRÜCKE and SUNDBERG are based upon this property. PEKELHARING makes use of a prolonged dialysis and precipitation with 0.2 p. m. HCl.

Very permanent pepsin solutions, from which the enzyme with considerable protein can be precipitated by alcohol, may be prepared by extraction with glycerin. Solutions having a strong action may also be prepared by making an infusion of the gastric mucosa of an animal in acidified water (2–5 p. m. HCl). This is unnecessary, as we can obtain pure gastric juice according to PAWLOW's method, and also because very active commercial preparations of pepsin can be bought in the market.

The Action of Pepsin on Proteins. Pepsin is inactive in neutral or alkaline reactions, but in acid liquids it dissolves coagulated protein bodies. The protein always swells and becomes transparant before it dissolves. Unboiled fibrin swells up in a solution containing 1 p. m. HCl, forming a gelatinous mass, and does not dissolve at ordinary tem-

[1] Zeitschr. f. physiol. Chem., 56. [2] *Ibid.*, 54.

perature within a couple of days. Upon the addition of a little pepsin, however, this swollen mass dissolves quickly at ordinary temperatures. Hard-boiled egg albumin, cut in thin pieces with sharp edges, is not perceptibly changed by dilute acid (2–4 p. m. HCl) at the temperature of the body in the course of several hours. But the simultaneous presence of pepsin causes the edges to become clear and transparent, blunt and swollen, and the protein gradually dissolves.

From what has been said above in regard to pepsin, it follows that proteins may be employed as a means of detecting pepsin in liquids. Ox-fibrin may be employed as well as coagulated egg albumin, which latter is used in the form of slices with sharp edges. As the fibrin is easily digested at the normal temperature, while the pepsin test with egg albumin requires the temperature of the body, and as the test with fibrin is somewhat more delicate, it is often preferred to that with egg albumin. When we speak of the "*pepsin test*" without further explanation we ordinarily understand it as the test with fibrin.

This test, nevertheless, requires care. The fibrin used should be ox-fibrin and not pig-fibrin, which last is dissolved too readily with dilute acid alone. The unboiled ox-fibrin may be dissolved by acid alone without pepsin, but this generally requires more time. In testing with unboiled fibrin at normal temperature, it is advisable to make a control test with another portion of the same fibrin with acid alone. Since at the temperature of the body unboiled fibrin is more easily dissolved by acid alone, it is best always to work with boiled fibrin.

As pepsin has not thus far been prepared in a positively pure condition, it is impossible to determine the absolute quantity of pepsin in a liquid. It is possible only to compare the relative amounts of pepsin in two or more liquids, which may be done in several ways.

The older method, that of BRÜCKE, consists in diluting the two pepsin solutions to be compared with certain proportions of 1 p. m. hydrochloric acid, so that when the amount of pepsin contained in the original solution is equal to 1, each solution contains a degree of dilution, p, corresponding to 1, $\frac{1}{2}$, $\frac{1}{4}$, $\frac{1}{8}$, $\frac{1}{16}$, etc. A flock of fibrin or a piece of hard-boiled egg is added to each test and the time noted when each test begins to digest and when it ends. The relative amount of pepsin is calculated from the rapidity of digestion as follows: The tests $p = \frac{1}{2}$, $\frac{1}{8}$, $\frac{1}{16}$, of one series is digested in the same time as tests $p = 1$, $\frac{1}{2}$, $\frac{1}{4}$ of the other series, hence the first solution contained four times as much pepsin. GRÜTZNER [1] has improved this test by using fibrin colored with carmine, and on comparing with carmine solutions of known dilution he determines colorimetrically the rapidity of digestion.

METT'S Method. Draw up white of egg in a glass tube 1–2 millimeters in diameter, coagulate it by plunging it into water at 95° C., and cut the ends off sharply; then add two tubes to each test-tube with a few cubic centimeters of the acid pepsin solution; allow them to digest at body temperature, and after a certain time, generally, after ten hours, measure the lineal extent of the digested

[1] Grützner, Pflüger's Arch., 8 and 106. See also A. Korn, "Ueber Methoden Pepsin quantitativ zu bestimmen," Inaug.-Dissert., Tübingen, 1902.

layer of albumin in the various tests, bearing in mind that the digested layer at each end must not be longer than 6–7 millimeters. The quantity of pepsin in the comparative tests is as the square of the millimeters of the albumin-column dissolved in the same time. Thus if in one case 2 millimeters of albumin were dissolved and in the other 3 millimeters, then the quantity of pepsin is as 4:9. If the fluid removed from the stomach, which is rich in bodies having a disturbing influence upon pepsin digestion, is to be tested, then the liquid must be first properly diluted with hydrochloric acid (NIERENSTEIN and SCHIFF [1]).

Objections have been raised against these methods from several sides, and they are in fact very uncertain. HUPPERT and E. SCHÜTZ measure the relative quantities of pepsin from the amount of secondary proteoses formed under certain conditions. The proteoses were determined by the polariscope. J. SCHÜTZ determines the total proteose-nitrogen, and SPRIGGS [2] finds that the change in the viscosity is a measure of the amount of pepsin.

VOLHARD and LÖHLEIN [3] use an acid casein solution for the pepsin determination, and determine, after precipitation with sodium sulphate, the acidity of the filtrate of the digested test as well as of the original control solution. The casein is precipitated as an acid compound by the sulphate, and the filtrate separated from the precipitate contains less acid than the original solution. In proportion as the digestion progresses less substance is precipitated by the sulphate, and the acidity of the filtrate becomes correspondingly higher. The increase in acidity in the different portions varies within certain limits as the square root of the quantity of ferment.

JACOBY suggested a method which is based on the fact that a cloudy solution of ricin becomes clear by the action of pepsin-hydrochloric acid, and indeed with varying rapidity with different quantities of pepsin. This method, which requires further testing, seems to be delicate and is of value, as is doubtless the following method of FULD and LEVISON.[4] This is based on the property that edestan can be precipitated from acid solution by NaCl, but not the proteoses formed therefrom.

A solution of 1 p. m. edestin in hydrochloric acid ($\frac{1}{10}$ normal) is prepared whereby the edestin is changed into edestan. The activity of a gastric juice (or a pepsin-hydrochloric acid solution) is tested in the following manner: the solution to be tested is placed in decreasing quantities in a series of test-tubes and allowed to act upon an equal quantity of the edestan solution, 2 cc., and the minimum of juice determined which is necessary to digest the solution, within one-half an hour and at room temperature, so that on the addition of solid NaCl and shaking no precipitate occurs. GROSS [5] suggested a similar method by using an acid casein solution and precipitating with sodium acetate.

The *rapidity of the pepsin digestion* depends on several circumstances. Thus *different acids* are unequal in their action; hydrochloric acid shows in slight concentration, 0.8–1.8 p. m., a more powerful action than any other acid, whether inorganic or organic. In greater concentration other acids may have a powerful action; but no constant relation has been found between the strength of various acids and their action in pepsin digestion, and the reports of the action of different acids are contradic-

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[1] Mett, see Pawlow, l.c.; 28; Nierenstein and Schiff, Berl. klin. Wochenschr., 40; Jastrowitz, Bioch. Zeitschr., 2.

[2] Huppert and Schütz, Pflüger's Arch., 80; J. Schütz, Zeitschr. f. physiol. Chem., 30; Spriggs, *ibid.*, 35.

[3] Hofmeister's Beiträge, 7.

[4] Jacoby, Bioch. Zeitschr., 1; Fuld and Levison, *ibid.* 6.

[5] Berl. klin. Wochenschr., 45.

tory.[1] Sulphuric acid, it seems, has a weaker action than the other inorganic acids. The *degree of acidity* is also of the greatest importance. With hydrochloric acid the degree of acidity is not the same for different protein bodies. For fibrin it is 0.8–1 p. m., for myosin, casein, and vegetable proteins about 1 p. m., for coagulated egg albumin, on the contrary, about 2.5 p. m. In regard to the dependence of the extent of transformation upon the quantity of enzyme and the time of digestion we refer to page 58. The *kind of protein* is of importance, for example, for besides what was said above in regard to the fibrin, hard-boiled egg albumin is much easier digested by an acidity of 1–2 p. m. HCl than liquid egg albumin, which is rather resistant to the action of gastric juice. The *accumulation of products of digestion* has a retarding action on digestion (page 65), although, according to CHITTENDEN and AMERMAN,[2] the removal of the digestion products by means of dialysis does not essentially change the relation between the proteoses and true peptones. Pepsin acts more slowly at low temperatures than it does at higher ones. It is even active in the neighborhood of 0° C., but with increasing temperature the rapidity of digestion also increases until about 40° C., when the maximum is reached. If the *swelling up of the protein* is prevented, as by the addition of neutral salts, such as NaCl, in sufficient amounts, or by the addition of bile to the acid liquid, digestion can be prevented to a greater or less extent. *Foreign bodies* of different kinds produce dissimilar effects, in which naturally the variable quantities in which they are added are of the greatest importance. Salicylic acid and carbolic acid, and especially sulphates (PFLEIDERER), retard digestion, while arsenious acid promotes it (CHITTENDEN), and hydrocyanic acid is relatively indifferent. Salts of the alkali and alkaline earth metals have a strong retarding action in strong concentration. By experiments with salt solutions so strongly diluted that the action, on account of the strong dissociation, was brought about by ions and not by the electrolytically neutral molecules (min. $\frac{1}{10}$ and max. $\frac{1}{4}$ normal salt solutions), J. SCHÜTZ[3] found that the anions had a much greater retarding action upon pepsin digestion than the cations. Of these latter the sodium cation had the strongest retarding action. Alcohol in large quantities (10 per cent and above) disturbs the digestion, while small quantities act indifferently. Metallic salts in very small quantities may indeed sometimes accelerate digestion, but otherwise

[1] See Wróblewski, Zeitschr. f. physiol. Chem., 21, and especially Pfleiderer, Pflüger's Arch., 66, which also gives references to other works; Larin, Biochem. Centralbl., 1, 484; and A. Pick, Wein. Sitzungsber., M. N. Klasse, 112.

[2] Journ. of Physiol. 14.

[3] Hofmeister's Beiträge, 5.

they tend to retard it. The action of metallic salts in different cases can be explained in various ways, but they often seem to form with proteins insoluble or difficultly soluble combinations. The alkaloids may also retard the pepsin digestion (CHITTENDEN and ALLEN [1]). A very large number of observations have been made in regard to the action of foreign substances on artificial pepsin digestion, but as these observations have not given any direct result in regard to the action of the same substances in natural digestion, as well as upon secretion and absorption, we will not discuss them here.

The Products of the Digestion of Proteins by Means of Pepsin and Acid. In the digestion of nucleoproteins or nucleoalbumins an insoluble residue of nuclein or pseudonuclein always remains, although under certain circumstances a complete solution may occur. Fibrin also yields an insoluble residue, which consists, at least in great part, of nuclein, derived from the form-elements inclosed in the blood-clot. This residue which remains after the digestion of certain proteins was called *dyspeptone* by MEISSNER. This name is therefore not only unnecessary but indeed erroneous, as this residue does not consist of bodies related to the peptones. In the digestion of proteins, substances similar to acid albuminates, *parapeptone* (MEISSNER [2]), *antialbumate*, and *antialbumid* (KÜHNE), may also be formed. On separating these bodies the filtered liquid, neutralized at boiling-point, contains *proteoses* and *peptones* in the old sense, while the so-called KÜHNE true peptone and the other cleavage products are obtained only after a longer and more intense digestion. The relation, between the proteoses, changes very much in different cases and in the digestion of the proteins. For instance, a larger quantity of primary proteoses is obtained from fibrin than from hard-boiled egg albumin or from the proteins of meat; and the different proteins, according to the researches of KLUG,[3] yield on pepsin digestion unequal quantities of the various digestive products. In the digestion of unboiled fibrin an intermediate product may be obtained in the earlier stages of the digestion—a globulin which coagulates at 55° C. (HASEBROEK [4]). For information in regard to the different proteoses and peptones which are formed in pepsin digestion see pages 127 to 136.

Action of Pepsin-Hydrochloric Acid on Other Bodies. The *gelatin-forming substances* of the connective tissue, of the cartilage, and of the

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[1] Studies from the Lab. Physiol. Chem. Yale University, **1**, 76. See also Chittenden and Stewart, *ibid.* **3**, 60.

[2] The works of Meissner on pepsin digestion are found in Zeitschr. f. rat. Med., **7**, **8**, **10**, **12**, and **14**.

[3] Pflüger's Arch., **65**.

[4] Zeitschr. f. physiol. Chem., **11**.

bones, from which last the acid dissolves only the inorganic substances, is converted into *gelatin* by digesting with gastric juice. The gelatin is further changed so that it loses its property of gelatinizing and is converted into gelatoses and peptone (see page 120). True *mucin* (from the submaxillary) is dissolved by the gastric juice, yielding substances similar to peptone, and a reducing substance similar to that obtained by boiling with a mineral acid. *Mucoids* from tendons, cartilage, and bones dissolve, according to POSNER and GIES,[1] in pepsin-hydrochloric acid, but leave a residue which amounts to about 10 per cent of the original material and which, as it seems, consists in great part, if not entirely, of a combination of proteid with glucothionic acid (Chapters VI and VII). The solution contains primary and secondary mucoproteoses and mucopeptones. The former contain glucothionic acid, but the latter do not. *Elastin* is dissolved more slowly and yields the previously mentioned substances (page 117). *Keratin* and the epidermal formations are insoluble. The *nucleins* are dissolved with difficulty, and the cell nuclei, therefore, remain in great part undissolved in the gastric juice. According to LONDON[2] and his collaborates the nucleic acids are not attacked in the stomach. The *animal cell-membrane* is, as a rule, more easily dissolved the nearer it stands to elastin, and it dissolves with greater difficulty the more closely it is related to keratin. The *membrane of the plant-cell* is not dissolved. *Oxyhæmoglobin* is changed into hæmatin and protein, the latter undergoing further digestion. It is for this reason that blood is changed into a dark-brown mass in the stomach. The gastric juice does not act upon *fat*, but, on the contrary, dissolves the cell-membrane of fatty tissue, setting the fat free. Gastric juice has no action on starch or the simple varieties of sugar. The statements in regard to the ability of gastric juice to invert cane-sugar are very contradictory. At least this action of the gastric juice is not constant, and if it is present at all, it is probably due to the action of the acid.

Pepsin alone, as above stated, has no action on proteins, and an acid of the intensity of the gastric juice can only very slowly, if at all, dissolve coagulated albumin at the temperature of the body. Pepsin and acid together not only act more quickly, but qualitatively they act otherwise than the acid alone, at least upon dissolved protein. This has led to the assumption of the presence of a *pepsin-hydrochloric acid* whose existence and action are only hypothetical. As pepsin digestion, it seems, yields finally the same products as the hydrolytic cleavage with acids, we can say for the present only that this enzyme acts like other catalysts in very powerfully accelerating a process which would also proceed without the catalyte.

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[1] Amer. Journ. of Physiol., **11**.

[2] Zeitschr. f. physiol. Chem. **70**, **72**.

Rennin or CHYMOSIN is the enzyme, which is especially characterized by the fact that it coagulates milk or casein solutions containing lime in neutral or indeed faintly alkaline reaction. It must probably be considered as a proteolytic enzyme. Rennin is habitually found in the neutral, watery infusion of the fourth stomach of the calf and sheep, especially in an infusion of the fundus part. In other mammals and in birds it is seldom found, and in fishes hardly ever in the neutral infusion. In these cases, as in man and the higher animals, a rennin-forming substance, a *rennin zymogen*, occurs, which is converted into rennin by the action of an acid (HAMMARSTEN). HEDIN has obtained a retarding solution by treating a neutral infusion of the stomachs of various animals with dilute ammonia and then neutralizing. These solutions entirely or partly retard the action of the rennin from the same animal and is destroyed by acid with the setting free of rennin. HEDIN therefore considers the rennin zymogen as a combination between rennin and an inhibitory substance, in which combination the inhibitory body is destroyed by treatment with acid; consequently the rennin appears in an active form.

According to BANG the rennin of the human and pig stomachs differs from that of the calf's stomach in being much more resistant to acids, more easily destroyed by alkalies, and that its action is much more accelerated by calcium chloride than that from the calf's stomach.[1] Active rennin occurs in the human stomach under physiological conditions, but may be absent under special pathological conditions.[2]

According to the experience of HAMMARSTEN the rennin of the pike and of the dog differs from that of the calf, and HEDIN [3] finds in the specific kind of inhibitory action of rennin produced by means of ammonia treatment as well as by immune serum, a proof that the rennin enzyme of different kinds of animals differ more or less from each other. In regard to this inhibition see pages 62–64.

Enzymes having a rennin action has also been found in the blood and several organs of higher animals as well as in invertebrates. Similar enzymes are also very widely distributed in the plant kingdom and numerous micro-organisms have the ability to produce rennin.

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[1] Deutsch. med. Wochenschr., 1899, and Pflüger's Arch., **79**.

[2] Schumburg, Virchow's Arch., **97**. A good review of the literature may be found in Szydlowski, Beiträge zur Kenntnis des Labenzym nach Beobachtungen an Säuglingen, Jahrb. f. Kinderheilkunde (N. F.), **34**. See also Lörcher, Pflüger's Arch., **69**. which also contains the pertinent literature. An excellent review of the literature on rennin and its action may be found in E. Fuld, Ergebnisse der Physiol., **1**, Abt. 1, 468.

[3] Hammarsten, Upsala Läkaref. förh. 8, 78 (1872). Zeitschr. f. physiol. Chem. **56**, 18 (1908), **68**, 119 (1910); Hedin, *ibid.* **72**, 187, **74**, 242, **76**, 355 (1911), **77**, 229 (1912).

The law given on page 58 in which the time of coagulation is inversely proportional to the amount of enzyme, is true for calf rennin (FULD [1]) and for sheep rennin (HEDIN [2]). The other rennins investigated do not follow this law at 37° C., which, according to VAN DAM, is due in the case of the pig rennin to its less resistance toward the alkali of the milk.[3]

Rennin is just as difficult to prepare in a pure state as the other enzymes. The purest rennin enzyme thus far obtained did not give the ordinary protein reactions. On heating its solution rennin is more or less quickly destroyed, depending upon the length of heating and upon the concentration. If an active and strong infusion of the gastric mucosa of the calf's stomach in water containing 3 p. m. HCl is heated to 40–45° C. for 48 hours, the rennin or nearly all, is destroyed, while the pepsin remains. A pepsin solution free from rennin can be obtained in this way.

A much-discussed question is, whether the digestion of protein and the rennet action are brought about by two special enzymes, or represent two different enzyme actions, or whether there is only one enzyme, the pepsin, which has both actions. The supporters of this last view dispose of the question in different ways. Some, like PAWLOW and PARASTSCHUK, consider the rennet action simply as the reverse of the synthetical action of pepsin, a view which is improbable in the highest degree. Others, such as SAWJALOW [4] and GEWIN, consider, on the contrary, that the coagulation of milk is only a pepsin action and indeed as the first step in the beginning of proteolysis, namely, the beginning of peptic digestion of casein. ROKOCZY [5] believes in the presence of two enzymes in the calf's stomach, one of which, the rennin, disappears on the increasing age of the animal.

The simultaneous occurrence in the animal and plant kingdoms of enzymes having a proteolytic and rennet action and the parallelism of the pepsin and rennet action indicates an identity of both enzymes and enzyme actions. This parallelism in fact does not prove much, because it has mostly been studied in acid reaction, while rennet is characteristically active in neutral or faintly alkaline reaction.

At the same time HAMMARSTEN [6] finds that in acid reactions no

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[1] Hofmeister's Beiträge 2.

[2] Not published investigations.

[3] Zeitschr. f. physiol. Chem. 64, 316 (1910).

[4] The recent literature on this question can be found in Hammarsten, Zeitschr. f. physiol. Chem., 56, 18 (1909).

[5] *Ibid.* 68, 421 (1910), 73, 453 (1911).

[6] Zeitschr. f. physiol. Chem. 68, 119 (1910), which also contains the recent literature.

parallelism exists in the two enzyme actions with extracts of the dog's and calf's, stomach, and, also on testing the two enzyme actions upon the same casein solution no parallelism was present. The pathological cases in man, if the observations are reliable, where only one enzyme action occurs, seems to dispute the identity of the action of these two enzymes. This opposition is also shown by the fact that pepsin, so far as known, only has a digestive action in the presence of free H ions, while the coagulation of milk occurs in the absence of these and indeed in the presence of HO ions. Among other facts which contravene the identity is the fact that a pepsin solution can be prepared which has a digestive action but cannot coagulate milk, and the reverse, namely, rennet solutions can be made which coagulate milk but do not have digestive action in acid reaction (HAMMARSTEN[1]). The observations of DUCCESCHI,[2] that pepsin but no rennin occurs in the stomach of the Didelphys, also conflict with the identity of the two enzymes.

The views of NENCKI and SIEBER[3] take a certain reconciliary position. According to them pepsin forms a gigantic molecule which has various side-chains, one of which has digestive action in acid solution while the others coagulate milk. This view coincides well with most of the observations made thus far.

In regard to the formation of *plasteins* under the influence of rennin solutions and other enzyme solutions, see Chapters I and II.

Gastric Lipase (STOMACH STEAPSIN). F. VOLHARD[4] made the discovery that the gastric juice has a strong fat-splitting action only when the fat is in a fine emulsion, as in the yolk of the egg, in milk or in cream. Considerable controversy has arisen in regard to the importance of the splitting of fat, and the occurrence of a special gastric lipase is indeed disputed. From numerous observations it follows without question that in man and many animals a gastric lipase occurs and is secreted with the gastric juice. Nevertheless the extent of fat splitting in the stomach is generally not very great. In its action this lipase follows SCHÜTZ'S rule and in its other properties it seems to vary in different animals.

The question whether the cover cells, principally, or the chief cells

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[1] Zeitschr. f. physiol. Chem., **56**.

[2] Centralbl., f. Physiol. **22**, 784.

[3] Zeitschr. f. physiol. Chem., **32**.

[4] Volhard, Münch. med. Wochenschr., 1900, and Zeitschr. f. klin. Med., **42**, **43**. See also Stade, Hofmeister's Beiträge, **3**; A. Fromme, *ibid.*, **7**; A Zinsser, *ibid.*; H. Engel., *ibid.*; and Inouye, Arch. f. Verdauungskrank., **9**; Falloise, Arch. internat. d. Physiol., **3** and **4**; London, Zeitschr. f. physiol, Chem., **50**; Levites, *ibid.*, **49**; Laqueur. Hofmeister's Beiträge, 8, 281; Heinsheimer, Deutsch. med. Wochenschr., **32**, and Arbeiten aus d. pathol. Institute, Berlin (Hirschwald, 1906).

also, or both, take part in the formation of free acid is disputed.[1] There can be no doubt that the hydrochloric acid of the gastric juice originates in the chlorides of the blood, because, as is well known, a secretion of perfectly typical gastric juice takes place in the stomachs of fasting animals or those which have starved for some time. As the chlorides of the blood are derived from the food, it is easily understood, as shown by CAHN,[2] that in dogs after a sufficiently long common-salt starvation, the stomach secreted a gastric juice containing pepsin, but no free hydrochloric acid. On the administration of soluble chlorides, a gastric juice containing hydrochloric acid was immediately secreted. The conditions are not so simple, because in the first case not only does the amount of hydrochloric acid diminish but, as shown by WOHLGEMUTH and then by KUDO, the quantity of juice diminishes greatly, and on the introduction of NaCl the quantity of juice secreted increases. According to PUGLIESE[3] the gastric juice in starvation, after a certain time, has a neutral reaction, and the introduction of NaCl does not now change its properties. In the secretion of free acid it is assumed by PUGLIESE that the gland cells, which decompose the chloride, have sufficient amounts of protein at their disposal. On the introduction of alkali iodides or bromides, KÜLZ, NENCKI and SCHOUMOW-SIMANOWSKI[4] have shown that the hydrochloric acid of the gastric juice is replaced by HBr, and to a less extent by HI. The secretion of free hydrochloric acid from the alkaline blood has been explained in various ways, but as yet no satisfactory theory has been suggested.[5]

In regard to the secretion of pepsin we must recall that this last is not already produced, but is formed from a preliminary step, a *pepsinogen* or *propepsin*. LANGLEY[6] has positively shown the existence of such a substance in the mucous coat. This substance, propepsin, shows a comparatively strong resistance to dilute alkalies (a soda solu-

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[1] See Heidenhain, Pflüger's Arch., 18 and 19, and Hermann's Handbuch, 5, part I, "Absonderungsvorgänge;" Klemensiewicz, Wien. Sitzungsber,. 71; Fränkel, Pflüger's Arch., 48 and 50; Contejean. l. c.; Kranenburg, Archives Teyler, Ser. II, Haarlem, 1901; and Mosse, Centralbl. f. Physiol., 17, 217; Fitzgerald, Proc. Roy. Soc. B. 82, 83; López-Suárez, Bioch. Zeitschr. 46, 490 (1912).

[2] Zeitschr. f. physiol. Chem., 10.

[3] Wohlgemuth, Arbeiten aus d. pathol. Institute, Berlin, 1906; Kudo, Bioch. Zeitschr. 16, 217 (1909), Pugliese, Maly's Jahresb., 36, 394.

[4] Külz, Zeitschr. f. Biologie, 23; Nencki and Schoumow, Arch. des sciences biol. de St. Pétersbourg, 3.

[5] Koeppe, Pflüger's Arch., 62; Benrath and Sachs, *ibid.*, 109; Maly, see v. Bunge's Lehrbuch der physiol. u. pathol. Chem., 4. Aufl., 1898; Schwarz, Hofmeister's Beiträge. 5,

[6] Schiff, Leçons, sur la physiol. de la digéstion, 1867, 2; Langley and Edkins, Journ. of Physiol., 7.

tion of 5 p. m.) which easily destroy pepsin (LANGLEY). Pepsin, on the other hand, withstands better than propepsin the action of carbon dioxide, which quickly destroys the latter. The occurrence of a rennin zymogen and possibly also of a steapsinogen, in the mucous coat has been mentioned above.

The question in what cells the two zymogens, especially the propepsin, are produced, has been extensively discussed for several years. Formerly, it was the general opinion that the cover cells were pepsin cells, but since the investigations of HEIDENHAIN and his pupils, LANGLEY and others, the formation of pepsin has been attributed to the chief cells.[1]

The Pyloric Secretion. That part of the pyloric end of the dog's stomach which contains no fundus glands was dissected by KLEMENSIEWICZ, one end being sewed together in the shape of a blind sac and the other sewed into the stomach. From the fistula thus created he was able to obtain the pyloric secretion of a living animal, later the secretion from a pyloric fistula has been obtained in other ways. This secretion is alkaline, viscous, jelly-like, rich in mucin, of a specific gravity of 1.009–1.010, containing 16.5–20.5 p. m. solids. It habitually contains pepsin, which has been proved by HEIDENHAIN by observations on a permanent pyloric fistula, and the amount may sometimes be considerable. CONTEJEAN investigated the pyloric secretion in other ways, and finds that it contains both acid and pepsin. The alkaline reaction of the secretions investigated by HEIDENHAIN and KLEMENSIEWICZ is due, according to CONTEJEAN, to an abnormal secretion caused by the operation, because the stomach readily yields an alkaline juice instead of an acid one under abnormal conditions. The reports of HEIDENHAIN and KLEMENSIEWICZ have nevertheless been substantiated by ÅKERMANN, KRESTEFF, SCHEMIAKINE and others.[2]

The secretion of gastric juice under different conditions may vary considerably. The statements concerning the quantity of gastric juice secreted in a certain time are therefore unreliable. ROSEMANN observed, on sham feeding in dogs, a secretion of 917 cc. in the course of 3½ hours—a considerable quantity. KUDO[3] found more pepsin in the secreted juice when the quantity of juice was less.

The Chyme and the Digestion in the Stomach. By means of the chemical stimulation caused by the food, a copious secretion of gastric

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[1] See footnote 1, p. 477.

[2] Heidenhain and Klemensiewicz, l. c.; Contejean, l. c., Chapter II, and Skand. Arch f. Physiol, 6; Åkermann, *ibid.*, 5; Kresteff, Maly's Jahresber., 30; Schemiakine Arch. des scienc. biolog. de St. Pétersbourg, 10.

[3] Rosemann, Pflüger's Arch. 118; Kudo, Bioch. Zeitschr. 16.

juice occurs, which gradually mixes with the swallowed food, and digests it more or less strongly. The material in the stomach during digestion, which has a pasty or thick consistency, and is called *chyme*, is not a homogeneous mixture of the ingesta with the various digestive fluids, gastric juice, saliva, and gastric mucus. but the conditions seem to be more complicated.

From the investigations of several workers,[1] on the movements of the stomach, we conclude that this organ in carnivora and also in man consists of two physiologically different parts, the pylorus and the fundus. The greater fundus part, which serves essentially as a reservoir, may be a rhythmic, strong contraction of the muscle, acting like a sphincter between it and the pylorus part, be separated from the latter, and according to some observers so completely so that during contraction scarcely anything passes from the fundus to the pylorus part. Differing from the fundus part the pylorus is the seat of very powerful contractions by which its contents are intimately mixed with gastric juice and are also driven through the pyloric valve into the intestine.

The contents of the pylorus part have an acid reaction, and a strong pepsin digestion takes place in the contents, which are thoroughly mixed with gastric juice. The contents of the fundus, on the contrary, show a different behavior, for here, as ELLENBERGER first showed, a special stratification of the various solid food-stuffs takes place.

By very instructive investigations on different animals (frogs, rats, rabbits, guinea-pigs, and dogs) GRÜTZNER[2] later showed that when the aminals are fed with food having different colors, and the stomach removed after a certain time, and the contents frozen, the frozen sections show a regular stratification of the contents. These layers are so arranged that the food first taken is found in direct contact with the mucosa, while the food taken later is enclosed by that partaken of first, and this prevents contact with the walls of the stomach. The empty stomach, whose walls touch each other, is so filled that, as a rule, the foodstuffs taken later are in the middle of the older food.

Because of this fact only the foodstuffs which lie close to the surface of the mucous membrane undergo the process of peptic digestion, and it is principally these ingesta, which lie on the surface and are laden with pepsin and mixed with gastric juice, which are shoved to the pylorus end, here mixed and digested, and finally moved into the intestine

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[1] Hofmeister and Schütz, Arch. f. exp. Path. u. Pharm., **20**; Moritz. Zeitschr. f. Biologie, **32**; Cannon, Amer. Journ. of Physiol., 1; Schemiakine, l. c.; Cathcart, Journ. of Physiol., 1911, **42**.

[2] See Ellenberger, Pflüger's Arch., **114**, and Scheunert, *ibid.*, **144**; Grützner, *ibid.*, **106**.

The fundus part is therefore less a digestion-organ than a storage-organ, and in the interior of the same, the food may remain for hours without coming in contact with a trace of gastric juice.

What has been said above applies at least to solid food. We have no extensive observations on the behavior of fluids or semifluid food. According to GRÜTZNER, in these cases, as well as in the above-mentioned experiments, the swallowed foodstuffs are not irregularly mixed together. Fluids quickly leave the stomach, which is also the case with a mixture of solid and fluid food.

Milk is an exception because it coagulates and the clot remains in the stomach while the whey quickly leaves the stomach.

The fact that only that part of the ingesta lying on the mucous membrane is mixed with gastric juice, while the mass in the interior is not acid in reaction, is of special importance for the digestion of starches in the stomach. By this we can explain why the salivary diastase, although sensitive toward acids, can continue its action for a long time in the contents of the stomach. That this is true was first found by ELLENBERGER and HOFMEISTER and then by CANNON and DAY[1] by special experiments upon animals. The occurrence of sugar and dextrin in the contents of the human stomach has been repeatedly observed. In carnivora, whose saliva shows scarcely any diastatic action, it is *a priori* not expected that there should be a diastatic action in the stomach, but the conditions are different in herbivora, where an abundant digestion of starch takes place in the various stomachs, according to the different species.

The gastric contents which have been prepared in the pylorus part are passed through the pylorus into the intestine intermittently. This material is generally fluid, but it is possible that pieces of solid food may also occur, and this has often been observed. Thin or plastic food leaves the stomach earlier than solid food, and it is obvious that the time in which the stomach unburdens itself depends naturally upon the coarseness or fineness of the food. This depends essentially upon the reflex action of the stomach or intestine, causing an opening or closing of the pylorus, which action is dependent upon the quantity and character of the food, the amount of fat, and the degree of acidity in the contents of the stomach and intestine. The emptying of the food into the small intestine causes, as shown by PAWLOW, a closing of the pylorus by chemo-reflex in which the hydrochloric acid and the fat take part, and we thus find in this regard an alternate action between the stomach and duodenum.

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[1] Ellenberger and Hofmeister, Maly's Jahresb., 15 and 16; Cannon and Day, Amer. Journ. of Physiol., 9.

This alternate action, according to CANNON[1] is due to the fact that the acid in the pylorus which acts upon the sphincter and makes possible the passage of the fluid chyme by the contraction of the muscles of the stomach. In the intestine the acid has a reverse stimulation upon the sphincter and causes a contraction of the same. As soon as the acid is neutralized the contractions of the sphincter cease and the passage of new portions of the chyme occur. If the flow of bile and pancreatic juice is prevented, and the neutralization of the acid contents of the stomach in the intestine is retarded, then the stomach does not eject its contents so often. The duration of gastric digestion varies according to conditions, and in consequence the reports of observers are widely divergent. BEAUMONT[2] found in his extensive observations on the Canadian hunter St. MARTIN that the stomach, as a rule, is emptied 1½–5½ hours after a meal, depending upon the character of the food.

The time in which different foods leave the stomach also depends upon their digestibility. Respecting the unequal digestibility in the stomach we must differentiate between the rapidity with which the foodstuffs are chemically transformed and that with which they leave the stomach and pass into the intestine. This distinction is especially important, and it is evident that the main factors governing speed of digestion and the time required before the food leaves the stomach are the kind of food and the fineness of its subdivision, and its action upon the gastric secretion, upon the pyloric reflexes, etc.

The observations of BOLDYREFF and others[3] on the action of fats and fatty acids and not too dilute hydrochloric acid (stronger than 0.2 per cent) are conclusive concerning the manner in which the properties of the food act upon the gastric secretion and upon the digestion in the stomach as a whole. Irrespective of the reducing action of the fats upon the extent and digestive power of the gastric juice BOLDYREFF found after food very rich in fat that the bile, pancreatic juice and intestinal juice migrate from the intestine into the stomach so that the digestion in the stomach in these cases is essentially brought about by the pancreatic juice.

We have numerous investigations on the rapidity with which the food is digested in the stomach of dogs, but we must especially mention

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[1] Amer. Journ. of Physiol., **20**.

[2] The Physiology of Digestion, 1833.

[3] Boldyreff, Pflüger's Arch., **121**, **140**; Migay, Maly's Jahresb. **39**; Best and Cohnheim, Zeitschr. f. Physiol. Chem. **69**; Cathcart, Journ. of Physiol. **42**. See also Abderhalden and Medigreceanu, Zeitschr. f. physiol. Chem., **57**.

the researches of E. ZUNZ,[1] LONDON[2] and his co-workers. LONDON, POLOWZOWA and SAGELMANN have observed that all the foodstuffs do not leave the stomach with the same rapidity, indeed, by feeding with bread (POLOWZOWA), the carbohydrates leave more quickly than the protein, and with a mixture of gliadin and beef-fat (SAGELMANN) the protein left the stomach more quickly than the fat. This is in accord with the recent observations of LONDON and SIVRÉ which show that the fats remain longest in the stomach, the starches the shortest and meat takes a middle position.[3] According to these authors the stomach has a sort of "selective capacity," but this is strongly disputed by SCHEUNERT and GRIMMER.[4] Nevertheless the researches of CANNON[5] on cats, making use of another method, have shown that this is true. After preliminary hunger the animals received different food, such as meat, fat, and carbohydrate mixed with bismuth subnitrate and then with the aid of the RÖNTGEN rays the time was noted when the food passed into the intestine. The carbohydrate leaves the stomach first, the proteins next, and the fats last. If the carbohydrate is given before the protein food, then it leaves the stomach with ordinary rapidity; while if protein food and then carbohydrate is given the passage of the carbohydrate is retarded. A mixture of protein food and carbohydrates leaves the stomach more slowly than carbohydrates alone, but faster than protein food alone. The fat, which remains in the stomach for a long time and leaves the stomach only in amounts which are absorbed or removed from the duodenum, retards the passage of the protein foods as well as the carbohydrates. TANGL and ERDÉLYI[6] have found in regard to the different kinds of fat, that a fat leaves the stomach the slower according to the height of its melting-point. According to LONDON and SCHWARZ,[7] with mixed protein feeding, the digestion in the stomach is regulated by that kind of protein, which, when alone, is removed from the stomach the slowest.

The reason why different foodstuffs leave the stomach with unequal rapidity is explained by CANNON by the above-mentioned action of the hydrochloric acid

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[1] E. Zunz, Hofmeister's Beiträge, **3**; Annal de la soc. roy. des scienc. med. Bruxelles **12**, **13**, and Mémoires publ. par l'Acad. roy. Belg., 1906, 1907, and 1908. Intern. Beitr. zu. Path. u. Ther. der Ernährungsstörungen **2**; Bull. de l'Acad. roy. de med. de Belgique, **24** (1910).

[2] The numerous works of London and co-workers will be found in Zeitschr. f. physiol. Chem., **45–53**, **55–58**, **60–74**.

[3] London with Polowzowa, Zeitschr. f. physiol. Chem., **49**, with Sagelmann, *ibid.*, **52**; London and Sivré, *ibid.* **60** (1909).

[4] Scheunert, Zeitschr. f. physiol. Chem., **51**; Grimmer, Bioch. Zeitschr., **3**.

[5] Amer. Journ. of Physiol., **12** and **20**; Amer. Journ. Med. Sciences, **138**, 504 (1909).

[6] Bioch. Zeitschr. **34**, 94 (1911).

[7] Zeitschr. f. physiol. Chem. **68**, (1910).

upon the pyloric sphincter. The proteins combine with the hydrochloric acid and hence its action upon the sphincter becomes weaker, while this is not the case with the carbohydrates. If the carbohydrates are moistened with alkali they leave the stomach more slowly than usual, and the acid proteins, on the contrary, leave the stomach earlier than other proteins.

As our knowledge of the digestibility of the different foods in the stomach is slight and uncertain, so also our knowledge of the action of other bodies, such as alcoholic drinks, bitter principles, spices, etc., on the natural digestion is very uncertain and imperfect. The difficulties which stand in the way of this kind of investigation are very great, and therefore the results obtained thus far are often ambiguous or conflict with each other. For example, certain investigators have observed that small quantities of alcohol or alcoholic drinks do not prevent but rather facilitate digestion; others observed only a disturbing action, while still others report having found that the alcohol first acts somewhat as a disturbing agent, but afterward, when it is absorbed, produces and abundant secretion of gastric juice, and thereby facilitates digestion. The accelerating action of alcohol upon the flow of gastric juice has been mentioned on page 464.

In regard to the importance of the stomach we used to be of the general opinion that an abundant peptonization of protein does not occur in the stomach, and that the food rich in protein is only chiefly prepared in the stomach for the real digestion in the intestine. That the stomach, at least the fundus, acts in the first place as a storage chamber, follows from the shape of this organ, especially in certain animals, and this function becomes especially prominent in certain newborn animals, as dogs and cats. In these animals the gastric secretion contains acid but no pepsin, and the casein of the milk is precipitated by the acid alone as solid lumps or as a solid coagulum filling the stomach. Gradually small quantities of this coagulum pass into the intestine and an overburdening of the intestine is thus prevented. In other animals, as the snake and certain fishes which swallow entire animals, the major part of the digestive work goes on in the stomach. The importance of the stomach for digestion cannot therefore be established in all instances. It varies in different animals and differs even in individual animals of the same species, depending upon the fineness or coarseness of the food, upon the greater or less rapidity with which peptonization takes place, and also upon the rapid or slow increment in the quantity of hydrochloric acid, etc.

In regard to the extent of chemical digestive work, i.e., in the first place the destruction of protein in the stomach, we have numerous researches, some carried out by the use of older methods and others by using newer and more reliable methods. Among these latter we must mention those of ZUNZ, LONDON and collaborators, TOBLER, LANG

and COHNHEIM.[1] These investigations refer to the conditions in dogs, and as shown by ROSENFELD[2] in horses, and by LÖTSCH[3] in pigs, that the conditions are different in other animals. The following description applies only to dogs.

In the dog ABDERHALDEN, LONDON and co-workers[4] have shown that in the stomach proteoses and peptones are formed, but no amino-acids, or at least not in any mentionable quantity. The scanty occurrence of amino-acids is substantiated by the observations of ZUNZ and others[5] that the amount of amino-nitrogen titratable with formol in the stomach contents, is only small.

In like manner we must agree in the belief that a part of the protein always leaves the stomach undigested and that the principal mass, about 80 per cent, passes into the intestine more or less digested. Besides this it also seems as if the peptones occur in the pylorus part to a greater extent than the proteoses, while in the fundus part the reverse is the case. Of the dissolved protein of the entire stomach-contents about 60 per cent exists as proteoses. Opinions are also contradictory in regard to the absorption of the decomposition products of the proteins in the stomach. While several investigators, like TOBLER, LANG, COHNHEIM, ZUNZ and others accept such an absorption, LONDON and co-workers positively deny this.

The digestion of sundry foods is not dependent on one organ alone, but is divided among several. For this reason it is to be expected that the various digestive organs can act for one another to a certain extent, and that therefore the work of the stomach could be taken up more or less by the intestine. This in fact is the case. Thus the stomachs of dogs and cats have been completely extirpated or nearly so (CZERNY, CARVALLO and PACHON, LONDON and collaborators), or that part necessary in the digestive process has also been eliminated by plugging the pyloric opening (LUDWIG and OGATA), and in both cases it was possible to keep the animal alive, well fed, and strong for a shorter or longer time. The extirpation of the stomach has also been repeatedly

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[1] Tobler, Zeitschr. f. physiol. Chem., 45; Lang, Bioch. Zeitschr., 2; Cohnheim, Münch. med. Wochenschr., 1907. In regard to the works of Zunz, London, and collaborators, see footnotes 1, 2 and 3, p. 482.

[2] Rosenfeld, Ueber die Eiweissverdauung im Magen des Pferdes, Inaug.-Dissert., Dresden, 1908.

[3] Lötsch. Zur Kenntnis der Verdauung von Fleisch im Magen und Dünndarm des Schweines, Inaug.-Dissert. Freiburg i. Sa., 1908; see also Abderhalden, Klingemann and Pappenhusen, Zeitschr. f. physiol. Chem. 71, 411 (1911).

[4] Abderhalden and London, with Kautsch, Zeitschr. f. physiol. Chem., 48, with L. Baumann, *ibid.*, 51, and with v. Körösy, *ibid.*, 51.

[5] Intern. Beitr. zu Path. u. Ther. d. Ern.-Stör. 2; London and Rabinowitsch, Zeitschr. f. physiol. Chem. 74.

performed on human beings with the same results.[1] In these cases it is evident that the digestive work of the stomach was taken up by the intestine; but all food cannot be digested in these cases to the same extent, and the connective tissue of meat especially is sometimes found to a considerable extent undigested in the excrements.

It is a well-known fact that the contents of the stomach may be kept without decomposing for some time by means of hydrochloric acid, while, on the contrary, when the acid is neutralized a fermentation commences by which lactic acid and other organic acids are formed. According to COHN, an amount of hydrochloric acid above 0.7 p. m. completely arrests lactic-acid fermentation, even under otherwise favorable circumstances, and according to STRAUSS and BIALOCOUR the limit of lactic-acid fermentation lies at 1.2 p. m. hydrochloric acid united to organic bodies. The hydrochloric acid of the gastric juice has unquestionably an antifermentative action, and also, like all dilute mineral acids, an antiseptic action. This action is of importance, as many pathogenic micro-organisms may be destroyed by the gastric juice. The common bacillus of cholera, certain streptococci, etc., are killed by the gastric juice, while others, especially as spores, are unacted upon. The fact that gastric juice can diminish or retard the action of certain toxalbumins, such as tetanotoxine and diphtheria toxine, is also of great interest (NENCKI, SIEBER, and SCHOUMOWA [2]).

Because of this antifermentative and antitoxic action of gastric juice it is considered that the principal importance of this juice lies in its antiseptic action. The fact that intestinal putrefaction is not increased on the extirpation of the stomach, as derived from experiments made on man and animal,[3] does not uphold this view.

Since the hydrochloric acid of the gastric juice prevents the contents of the stomach from fermenting, with the generation of gas, those *gases* which occur in the stomach probably depend, at least in great measure, upon the swallowed air and saliva, and upon those gases generated in the intestine and returned through the pyloric valve. PLANER found in the stomach-gases of a dog 66–68 per cent N, 23–33 per cent

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[1] Czerny, cited from Bunge, Lehrbuch d. physiol. u. path. Chem. 4. Aufl., Theil 2, 173; Carvallo and Pachon, Arch. d. Physiol. (5), 7; Ogata, Arch. f. (Anat. u.) Physiol. 1883; Grohé, Arch. f. exp. Path. u. Pharm. 49; London and collaborators, Zeitschr. f. physiol. Chem. 74, 328 (1911); in regard to the case in man, see Schlatter in Wróblewski, Centralbl. f. Physiol. 11, p. 665, and the surgical journals.

[2] Cohn, Zeitschr. f. physiol. Chem., 14; Strauss and Bialocour, Zeitschr. f. klin. Med., 28. See also Kühne, Lehrb., 57; Bunge, Lehrb. d. Physiol., 4. Aufl., 148 and 159; Hirschfeld, Pflüger's Arch., 47; Nencki, Sieber, and Schoumowa, Centralbl. f. Bacteriol., etc., 28. In regard to the action of gastric juice upon pathogenic microbes we must refer the reader to hand-books of bacteriology.

[3] See Carvallo and Pachon, l. c., and Schlatter in Wróblewski, l. c.

CO_2, and only a small quantity, 0.8–6.1 per cent, of oxygen. SCHIERBECK[1] has shown that a part of the carbon dioxide is formed by the mucous membrane of the stomach. The tension of the carbon dioxide in the stomach corresponds, according to him, to 30–40 mm. Hg in the fasting condition. It increases after partaking food, independently of the kind of food, and may rise to 130–140 mm. Hg during digestion. The curve of the carbon-dioxide tension in the stomach is the same as the curve of acidity in the different phases of digestion, and SCHIERBECK also found that the carbon-dioxide tension is considerably increased by pilocarpine, but diminished by nicotine. According to him, the carbon dioxide of the stomach is a product of the activity of the secretory cells.

After death, if the stomach still contains food, autodigestion goes on not only in the stomach, but also in the neighboring organs, during the slow cooling of the body. This leads to the question, Why does the stomach not digest itself during life? Ever since PAVY has shown that after tying the smaller blood-vessels of the stomach of dogs the corresponding part of the mucous membrane was digested, efforts have been made to find the cause in the neutralization of the acid of the gastric juice by the alkali of the blood. That the reason for the non-digestion during life is to be sought for in the normal circulation of the blood cannot be contradicted; but the reason is not to be found in the direct neutralization of the acid. The investigations of FERMI and OTTE[2] show that the blood circulation acts in an indirect manner by the normal nourishment of the cell protoplasm, and this is the reason why the digestive fluids, the gastric juice as well as the pancreatic juice, act differently upon the living protoplasm as compared with the dead. We know nothing about this resistance of the living protoplasm. Some claim that it is closely connected with occurrence of different inhibitory substances in the gastric mucosa. Of these the substance found by WEINLAND is thermolabile while that of DANILEWSKY, HÄNSEL and SCHWARZ is resistant toward heat.[3] Without mentioning the still unknown nature of these bodies, the neutral gastric juice, as well as an acid infusion of the mucosa, has such a strong digestive action that the inhibiting action of the mentioned substances can only be shown under special conditions, and it is therefore difficult to conceive how these substances could have a protective action in life.

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[1] Planer, Wien. Sitzungsber., **42**; Schierbeck, Skand. Arch. f. Physiol., **3** and **5**.

[2] Pavy, Phil. Transactions, **153**, Part I, and Guy's Hospital Reports, **13**; Otte, Travaux du laboratoire de l'Institut de Physiol. de Liège, **5**, 1896, which also contains the literature.

[3] Weinland, Zeitschr. f. Biologie, **44**; Hänsel, Biochem. Centralbl., **1**, p. **404**, and **2**, p. 326; Schwartz, Hofmeister's Beiträge, **6**.

Under pathological conditions irregularities in the secretion may occur. The quantity of enzymes may be diminished and both enzymes or, as found in certain cases, one (the rennin), may be absent. The hydrochloric acid may also be absent or may exist in very small amounts. A pathological high degree of acidity of the pure juice is not very probable, while on the contrary a hypersecretion of gastric juice in different forms does occur.

In testing the gastric juice or the filtered stomach contents, diluted with digestive hydrochloric acid, for *pepsin*, we make use of the pepsin tests given on pages 469, 470. In testing for *rennin* the liquid must be first carefully neutralized, and 1–2 cc. of this liquid added to 10 cc. milk. In the presence of appreciable quantities of rennin, the milk should coagulate at room temperature within 10–20 minutes without changing its reaction. The addition of lime salts is unnecessary, and may readily lead to erroneous conclusions.

In many cases it is especially important to determine the *degree of acidity of the gastric juice*. This may be done by the ordinary titration methods. Phenolphthalein must not be used as an indicator, as too high results are produced in the presence of large quantities of proteins. Good results may be obtained, on the contrary, by using very delicate litmus paper. Although the acid reaction of the contents of the stomach may be caused simultaneously by several acids, still the degree of acidity is here, as in other cases, expressed in only one acid, e.g., HCl. Generally the acidity is designated by the number of cubic centimeters of N/10 sodium hydroxide required to neutralize the several acids in 100 cc. of the liquid of the stomach. An acidity of 43 per cent means that 100 cc. of the liquid of the stomach required 43 cc. of N/10 sodium hydroxide to neutralize it.

It is also important to be able to ascertain the nature of the acid or acids occurring in the contents of the stomach. For this purpose, and especially for the *detection of free hydrochloric acid*, a great number of color reactions have been proposed which are all based upon the fact that the coloring substance gives a characteristic color with very small quantities of hydrochloric acid, while lactic acid and the other organic acids do not give these colorations, or only in a certain concentration, which can hardly exist in the contents of the stomach. These reagents are a mixture of FERRIC-ACETATE and POTASSIUM-SULPHOCYANIDE solutions (MOHR'S reagent has been modified by several investigators), METHYL-ANILINE-VIOLET, TROPÆOLIN 00, CONGO RED, MALACHITE-GREEN, PHLORO-GLUCINOL-VANILLIN, DIMETHYLAMINOAZOBENZENE, and others. As reagents for *free lactic acid*, UFFELMANN suggests a strongly diluted, amethyst-blue solution of FERRIC CHLORIDE and CARBOLIC ACID, or a strongly diluted nearly colorless solution of FERRIC CHLORIDE. These give a yellow color with lactic acid, but not with hydrochloric acid or with volatile fatty acids.

The value of these reagents in testing for free hydrochloric acid or lactic acid is still disputed. Among the reagents for free hydrochloric acid it seems STEENSMA'S [1] modification of GÜNZBURG'S test with phloroglucinol-vanillin, and

[1] Bioch. Zeitschrift, 8.

the test with tropæolin 00, performed at a moderate temperature as suggested by BOAS, and the test with dimethylaminoazobenzene, which is the most delicate, seem to be the most valuable. If these tests give positive results, then the presence of hydrochloric acid may be considered as proved. A negative result does not eliminate the presence of hydrochloric acid, as the delicacy of these reactions has a limit, and also the simultaneous presence of protein, peptones, and other bodies influences the reactions more or less. The reactions for lactic acid may also give negative results in the presence of comparatively large quantities of hydrochloric acid in the liquid to be tested. Sugar, sulphocyanides, and other bodies may act with these reagents like lactic acid.

In testing for lactic acid it is safest to shake the material with ether and test the residue after the evaporation of the solvent. On the evaporation of the ether the residue may be tested in several ways. BOAS utilizes the property possessed by lactic acid of being converted into aldehyde and formic acid on careful oxidation with sulphuric acid and manganese dioxide. The aldehyde is detected by its forming iodoform with an alkaline iodine solution or by its forming aldehyde-mercury with NESSLER'S reagent. CRONER and CRONHEIM [1] have suggested another method.

The quantitative estimation consists in the formation of iodoform with N/10 iodine solution and caustic potash, adding an excess of hydrochloric acid and titrating with a N/10 sodium-arsenite solution, and retitrating with iodine solution, after the addition of starch-paste, until a blue coloration is obtained. This method presupposes the use of ether entirely free from alcohol. For details see the original publication and the modification of this method suggested by JERUSALEM.[2]

In order to be able to judge correctly of the value of the different reagents for free hydrochloric acid, it is naturally of greatest importance to be clear in regard to what we mean by free hydrochloric acid. It is a well-known fact that hydrochloric acid combines with proteins, and a considerable part of the hydrocholoric acid may therefore exist in the contents of the stomach, after a meal rich in proteins, in combination with them. This hydrochloric acid combined with proteins cannot be considered as free, and it is for this reason that certain investigators consider such methods as those of SJÖQVIST, which will be described below, as of little value. However, it must be remarked that, according to the unanimous experience of many investigators, the hydrochloric acid combined with proteins is physiologically active and in this regard we must refer to the recent investigations of ALB. MÜLLER and J. SCHÜTZ.[3] Those reactions (color reactions) which only respond to actually free hydrochloric acid do not show the physiologically active hydrochloric acid. The suggestion of determining the "physiologically active" hydrochloric acid instead of the "free" seems to be correct in principle; and as the conceptions of free and of physiologically active hydrochloric acid are not the same, it must always be well defined whether one wishes to determine the actually free or the physiologically active hydro-

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[1] Boas. Deutsch. med. Wochenschr., 1893, and Münchener med. Wochenschr. 1893, Croner and Cronheim, Berl. klin. Wochenschr., 1905. See also Thomas, Zeitschr. f. physiol. Chem., 50.

[2] Bioch. Zeitschr., 12.

[3] Alb. Müller, Deutsch. Arch. f. klin. Med., 88, and Pflüger's Arch., 116; J. Schütz. Wien, klin. Wochenschr., 20, and Wien. med. Wochenschr., 1906 (older literature).

chloric acid before any conclusions are drawn as to the value of a certain reaction.

The acid reaction may be partly due to free acid, partly to acid salts (monophosphates), and partly to both. According to LEO,[1] one can test for acid phosphates by calcium carbonate, which is not neutralized therewith, while the free acids are. If the gastric content has a neutral reaction after shaking with calcium carbonate, and the carbon dioxide is driven out by a current of air, it contains only free acid; if it has an acid reaction, acid phosphates are present, and if it is less acid than before, it contains both free acid and acid phosphate. It must not be forgotten that a faint acid reaction may, after treatment with calcium carbonate, also be due to the protein. This method can likewise be applied in the estimation of free acid.

Various titration methods have been suggested for the estimation of the free hydrochloric acid, but these cannot yield conclusive results for the reasons given in Chapter I. For this determination physico-chemical methods (page 74), are necessary, but they have not been used to any great extent for clinical purposes. HOLMGREN[2] has suggested a method for estimating hydrochloric acid based upon the adsorption phenomenon.

A great number of methods have been suggested for the quantitative estimation of the total acidity, among which we must mention those of K. MÖRNER and SJÖQVIST, which are extensively used. As the value of a special determination of the free and total hydrochloric acid is doubtful, or at least disputed, and also as the question is chiefly of clinical interest we must refer to the hand-books of clinical investigations of V. JAKSCH, EULENBURG, KOLLE, and WEINTRAUD and of SAHLI. The same applies to the tests for volatile fatty acids.

III. THE GLANDS OF THE MUCOUS MEMBRANE OF THE INTESTINE AND THEIR SECRETIONS.

The Secretion of Brunner's Glands. These glands are partly considered as small pancreatic glands and partly as mucous or salivary glands. Their importance is not the same in all animals. According to GRÜTZNER they are in dogs closely related to the pyloric glands and contain pepsin. This also coincides with the observations of GLAESSNER and of PONOMAREW, which differ from each other only in that PONOMAREW finds that the secretion is inactive in alkaline reaction and contains only pepsin, while GLAESSNER claims it is active in both acid and alkaline reaction and that it contains pseudopepsin. According to ABDERHALDEN and RONA the pure duodenal secretion of the dog contains a proteolytic enzyme which does not belong to the trypsin type but rather to the pepsin variety. The statements as to the occurrence of a diastatic enzyme in BRUNNER'S glands are disputed. SCHEUNERT and GRIMMER[3] indeed found diastatic enzyme in the duodenal glands of the horse, ox, pig and rabbit, but no proteolytic or rennin enzyme.

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[1] Centralbl, f. d. med. Wissensch., 1889, p. 481; Pflüger's Arch., 48, and Berlin. klin. Wochenschr., 1905, p. 1491.

[2] Deutsch. med. Wochenschr., 1911, p. 247.

[3] Grützner, Pflüger's Arch., 12; Glaessner, Hofmeister's Breiträge, 1; Ponomarew; Biochem. Centralbl., 1, 351; Abderhalden and Rona, Zeitschr. f. physiol. Chem., 47; Scheunert and Grimmer, cited in Bioch. Centralbl., 5, 673.

The Secretion of Lieberkuhn's Glands. The secretion of these glands has been studied with the aid of a fistula in the intestine according to the method of THIRY and VELLA or of PAWLOW. According to BOLDYREFF,[1] in dogs, with an empty stomach, a scanty secretion lasting about 15 minutes occurs at regular intervals for about two hours. According to BOLDYREFF the intestinal juice is obtained from a THIRY-VILLA fistula outside of the digestion period without any apparent stimulation. According to this experimenter, during gastric digestion the juice is periodically but less abundantly secreted as the time interval is much longer, namely three, four or five hours. Otherwise it is generally admitted that the partaking of food causes the secretion, or if this is continuous, as in lambs (PREGL), it increases the secretion. The researches of DELEZENNE and FROUIN show without question that the passage of chyme into the intestine increases the secretion of the intestinal juice. The acid causes a formation of secretin (see below), and this produces, according to the above investigators, a secretion of intestinal juice. Among the chemically active substances causing a secretion we must mention acids in general and gastric juice. Soaps, chloral, ether and on intravenous injection, also intestinal juice or an extract of the intestinal mucosa (FROUIN), are chemical excitants of intestinal juice. Several salts, NaCl, Na_2SO_4, and others, may cause an abundant secretion of fluid into the intestine when injected intravenously or subcutaneously, as well as after direct application to the peritoneal surface of the intestine. This action can be arrested by the antagonistic, inhibiting action of a lime salt (MACCALLUM). Pilocarpine, which has the power of increasing the activity of secretions, does not increase the secretion in lambs, and in dogs it does not seem to be always active (GAMGEE[2]).

Mechanical irritation of the intestinal mucosa increases the secretion in dogs (THIRY) as well as in man (HAMBURGER and HEKMA), but it is still doubtful whether we here have a perfectly physiological juice. In the cases observed by HAMBURGER and HEKMA[3] the flow of fluid was greatest at night as well as between five and eight o'clock in the afternoon, and was lowest between two and five o'clock in the afternoon. The quantity of this secretion in the course of twenty-four hours has not been exactly determined.

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[1] Thiry, Wien, Sitz.-Ber., **50**; Vella, Molleschott's Untersuch., **13**; Boldyreff, Zeitschr. f. physiol. Chem., **50**, Centralbl. f. Physiol. **24**, 93 (1910).

[2] Delezenne and Frouin, Compt. rend. soc. biol., **56**; Frouin, *ibid.*, **56** and **58**; MacCallum, University of California Publications, 1, 1904; Gamgee, Physiol. Chemistry, 2, 410 (literature).

[3] Journ. de Physiol. et d. path. gén., 1902 and 1904.

According to DELEZENNE and FROUIN, if any mechanical irritation is prevented, the fluid flowing spontaneously from a fistula in a dog is ten times more abundant in the duodenum than that in the middle or lower part of the jejunum. In the upper part of the small intestine of the dog, on the contrary, this secretion is scanty, slimy, and gelatinous; in the lower part it is more fluid, with gelatinous lumps or flakes (RÖHMANN). Intestinal juice has a strong alkaline reaction toward litmus, generates carbon dioxide on the addition of an acid, and contains (in dogs) nearly a constant quantity of NaCl and Na_2CO_3, 4.8–5 and 4–5 p. m. respectively (GUMILEWSKI, RÖHMANN[1]). The intestinal juice of the lamb corresponded to an alkalinity of 4.54 p. m. Na_2CO_3. It contains protein (THIRY found 8.01 p. m.), the quantity decreasing with the duration of the elimination. The quantity of solids varies. In dogs the quantity of solids is 12.2–24.1 p. m. and in lambs 29.85 p. m. The specific gravity of the intestinal juice of the dog, according to the observations of THIRY, is 1.010–1.0107, and in lambs 1.0143 (PREGL). The intestinal juice from lambs contains 18.097 p. m. protein, 1.274 p. m. proteoses and mucin, 2.29 p. m. urea, and 3.13 p. m. remaining organic bodies.

We have the investigations of DEMANT, TURBY and MANNING, H. HAMBURGER and HEKMA and NAGANO[2] on the human intestinal juice. Human intestinal juice has a low specific gravity, nearly 1.007, about 10–14 p. m. solids, and is strongly alkaline toward litmus. The content of alkali calculated as sodium carbonate is 2.2 p. m., according to NAGANO, HAMBURGER and HEKMA, and 5.8–6.7 p. m. Na Cl. The determination of the freezing-point was −0.62° (HAMBURGER and HEKMA).

The intestinal juice of the dog contains, according to BOLDYREFF a *lipase* which acts especially upon emulsified fat (milk), and is different from pancreas lipase, in that its action is not accelerated by bile. JANSEN[3] found that the lipase was secreted from a THIRY-VELLA fistula especially under the influence of bile and acid. The intestinal juice of animals and man also contains an enzyme, *erepsin*, discovered by O. COHNHEIM, which does not ordinarily have a splitting action upon native proteins, but upon proteoses and peptones. It also possibly contains a *nuclease*, and it also has a faint *amylolytic action*. The juice, and to a high degree the mucous coat, contains *invertase* and *maltase*, which

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[1] Gumilewski, Pflüger's Arch., 39. Röhmann, *ibid.*, 41.

[2] Demant, Virchow's Arch., 75; Turby and Manning, Centralbl. f. d. med. Wissenschaft, 1892, 945; Hamburger and Hekma, l. c.; Nagano, Mitt. aus d. Grenzgeb. d. Med. u. Chir., 9.

[3] Boldyreff, Archiv. d. sciences biolog, de St. Pétersbourg, 11; Jansen, Zeitschr. f. physiol. Chem. 58, 400 (1910).

fact has been substantiated by the observations of PASCHUTIN, BROWN and HERON, BASTIANELLI, and TEBB.[1] A lactose-inverting enzyme, a *lactase*, also occurs, as shown by RÖHMANN and LAPPE, PAUTZ and VOGEL, WEINLAND, and ORBAN,[2] in new-born infants and young animals, and also in grown mammals which were fed upon a milk diet (see Chapter I, page 52). The lactase can be obtained more abundantly from the mucosa than from the juice and according to some occurs only in the cells. The claims as to the occurrence of a glucoside splitting enzyme are disputed (FROUIN, OMI [3]).

Besides erepsin and the other enzymes mentioned, the intestinal mucosa also contains substances which have an inhibitory action upon pepsin and trypsin. (DANILEWSKY and WEINLAND [4]), also *enterokinase* or a mother-substance of the same, and finally also the so-called *prosecretin*. These two last-mentioned bodies, which are closely connected with the secretion of pancreatic juice, will be discussed in connection with this digestive fluid.

The various enzymes are not formed in equal quantities in all parts of the intestine. Diastase and invertase occur, according to BOLDYREFF, all through the intestine, while the lipase on the contrary does not occur in the lower parts. The kinase occurs only in the upper part of the intestine (BOLDYREFF, BAYLISS and STARLING, DELEZENNE). According to HEKMA the kinase occurs in all parts of the intestine, but most abundantly in the duodenum and the upper part of the jejunum. The enzymes, FALLOISE claims, generally occur in greatest abundance in the upper parts of the intestine; but the erepsin occurs to a greater extent in the jejunum than in the duodenum. According to the investigations of VERNON the behavior of erepsin is not the same in different animals. In cats and hedge-hogs the duodenum is richer in erepsin than the jejunum and ileum; in rabbits it is the reverse, namely, the ileum is much richer than the duodenum. The secretin, according to BAYLISS and STARLING, is formed entirely in the upper part of the intestine. The epithelium-cells of the glands or the mucous membrane are generally considered as the seat of formation of the enzymes, and the same is true also for the enterokinase, according to BAYLISS and STARLING,

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[1] Paschutin, Centralbl. f. d. med. Wissensch., 1870, 561; Brown and Heron, Annal. d. Chem. u. Pharm., **204**; Bastianelli, Moleschott's Untersuch, zur Naturlehre, **14** (this contains all the older literature). See also Miura, Zeitschr. f. Biologie, **32**; Widdicombe, Journ. of Physiol., **28**; Tebb, *ibid.*, **15**.

[2] Röhmann and Lappe, Ber. d. deutsch, chem. Gesellch., **28**; Pautz and Vogel, Zeitschr. f. Biologie. **32**; Weinland, *ibid.*, **38**; Orban, Maly's Jahresber., **29**.

[3] Frouin and Thomas, Arch. internat. de Physiol., 7; Omi, Das Verhalten des Salizins im tierschen Organismus, Inaug.-Dissert. Breslau, 1907.

[4] See footnote 3, p. 486.

HEKMA, FALLOISE, and others, which, however, DELEZENNE says,[1] is formed in the leucocytes and PEYER'S glands.

Erepsin. This enzyme, discovered by O. COHNHEIM, has no direct action upon native proteins with the exception of casein, but has the power of splitting proteoses, peptones and certain polypeptides. In this change mono- as well as diamino-acids are produced. Erepsin occurs in the mucous membrane and in the intestinal juice of man as well as of dogs; the mucous membrane seems to be richer than the juice (SALASKIN, KUTSCHER and SEEMANN[2]). An enzyme like erepsin also occurs in the pancreas (BAYLISS and STARLING, VERNON), and this has the power of acting upon casein, but not, or only faintly, upon fresh fibrin. This erepsin is probably identical with the enzyme *nuclease*, discovered by F. SACHS in the pancreas, which acts upon nucleic acids, while NAKAYAMA claims that erepsin differs from trypsin in having a cleavage action upon nucleic acids. Intestinal erepsin is not inhibited, according to GLAESSNER and STAUBER, by blood-serum and differs in this regard from trypsin. Erepsin shows a great similarity to the intracellular enzymes active in autolysis, and according to VERNON and others erepsins occur in the various tissues of invertebrates as well as vertebrates. These tissue erepsins, which are closely related to the autolytic enzymes, if they are not identical with them, behave somewhat differently from the intestinal erepsin and are not identical therewith. Enzymes, having an action similar to erepsin, occur, VINES believes,[3] in all plants so far investigated.

Erepsin becomes inactive on heating to 59°. It works best in alkaline solution, but has hardly any action in faint acid reaction. In this regard, as well as by the fact that only a little ammonia is split off by its action upon peptone substances, it differentiates itself from certain of the autolytic enzymes studied so far. The optimum of alkalinity is, according to EULER,[4] at least in the splitting of a polypeptide, much lower than the optimum for tryptic digestion.

The secretion of the GLANDS IN THE LARGE INTESTINE seems to consist chiefly of mucus. Fistulas have also been introduced into these

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[1] Boldyreff, Arch. d. scienc. biolog, de St. Pétersbourg, **11**; Bayliss and Starling, Journ. of Physiol., **29**, **30**; Hekma, l. c.; Falloise, see Biochem. Centralbl., **4**, p. 153; Vernon, Journ. of Physiol., **33**; Delezenne, Compt. rend. soc. biolog., **54** and **56**.

[2] Cohnheim, Zeitschr. f. Physiol. Chem., **33**, **35**, **36**, and **47**; Salaskin, *ibid.*, **35**; Kutscher and Seemann, *ibid.*, **35**.

[3] Bayliss and Starling, Journ. of Physiol., **30**; Vernon, *ibid.*, **30** and **33**. See also Cohnheim and Pletnew, Zeitschr. f. physiol. Chem. **69**; F. Sachs, Zeitschr. f. physiol. Chem., **46**; Nakayama, *ibid.*, **41**; Glaessner and Stauber, Bioch. Zeitschr. **25**; Vines, Annals of Botany, **18**, **19**, and **23**.

[4] Zeitschr. f. physiol. Chem., **51**.

During starvation the secretion almost stops, but commences again after partaking of food and reaches its maximum, it is claimed by BERNSTEIN, HEIDENHAIN, and others, within the first three hours.

PAWLOW and his pupils, especially SCHEPOWALNIKOFF, have shown that the above-mentioned (page 492) enterokinase activates the trypsinogen into trypsin. These observations were later confirmed by others, by DELEZENNE and FROUIN, POPIELSKI, CAMUS and GLEY, BAYLISS and STARLING, ZUNZ, and have been further studied. The pure juice contains, at least as a rule, only trypsinogen, and no trypsin. By mixing with the intestinal juice, or by contact with the intestinal mucosa, the trypsinogen is converted into trypsin by the kinase. Enterokinase, which itself has no action upon proteins, and therefore is not a proteolytic enzyme, is not well known. It is made inactive by heating and is therefore considered by many (including PAWLOW) as an enzyme. Others, on the contrary, like HAMBURGER and HEKMA, DASTRE and STASSANO, deny the enzyme nature of enterokinase because they find that a certain quantity of intestinal juice will activate only a certain quantity of trypsin. Enterokinase has been found in man and all mammals investigated. According to most investigators it is formed in the glands or the cells of the intestinal mucosa, while according to DELEZENNE it comes from PEYER'S patches and from the lymph-glands and leucocytes, hence impure fibrin containing leucocytes acts as a kinase. These deductions of DELEZENNE are disputed by BAYLISS and STARLING, HEKMA and others.

If we accept the view that the juice secreted after partaking food is regularly free from trypsin, still under other circumstances the juice may contain trypsin. Thus, according to CAMUS and GLEY, the juice secreted under the influence of secretin (see below) is not always free from trypsin, and ZUNZ found that WITTE'S peptone or pilocarpine causes a secretion of juice which often contained trypsin and was directly active. According to CAMUS and GLEY not only does an exterior activation of the trypsinogen in the juice take place, but also in the interior of the gland. An auto-activation of the juice in certain cases is also accepted by others (SAWITSCH [1]).

The activation of the trypsinogen into trypsin may, in life, be brought about —as the researches of HERZEN, which have been substantiated by GACHET and PACHON, BELLAMY, MENDEL and RETTGER, have shown—not only in the intestine, but also in the gland itself. This activation of the trypsinogen in the gland itself is caused in a still undiscovered manner by a body of unknown nature formed in the spleen, which is congested during digestion. Such a " charging " of the pancreas

[1] Camus and Gley, Journ. de Physiol. et de Pathol, gén., 1907; Zunz, Recherches sur l'activation de sac pancreatique par les Sels., Bruxelles, 1907; Sawitsch, Zentralbl. f. d. ges. Physiol. u. Path. des Stoffwechsels, 1909.

by the spleen has been repeatedly suggested by SCHIFF,[1] but this has recently been denied by PRYM. According to this experimenter the extirpation of the spleen causes no change in the properties of the pancreatic juice, and the intravenous injection of spleen infusion is also without action on a splenectomized dog with permanent pancreatic fistula. The observations of HERZEN that a spleen infusion has a strong activating action upon a weak pancreas infusion were substantiated by PRYM,[2] but he claims that this is due essentially to micro-organsims. Besides this the spleen itself contains proteolytic enzymes (page 371).

The conversion of the trypsinogen into trypsin in the removed gland or in an infusion under the influence of air and water and also by other bodies has been known for a long time. According to VERNON the trypsin itself has a strong activating action upon trypsinogen, and in this regard it is more active than enterokinase. The correctness of this statement is still denied by BAYLISS and STARLING and by HEKMA. The ordinary view of HEIDENHAIN, that the transformation of trypsinogen into trypsin is also brought about by acids, has been found to be incorrect by HEKMA.[3] Besides the enterokinase and the micro-organisms, there are other activators of the trypsinogen. As first shown by DELEZENNE and then by ZUNZ, by further investigations the lime salts have a special power in activating trypsinogen.[4] These last do not act immediately, but only after some time, for example, a couple of hours, and then they activate suddenly. The lime salts are not necessary for the digestive action of the juice, and when the activation has once taken place, they can be removed without any harm. They probably have a similar action as in the coagulation of the blood. According to DELEZENNE the lime salts have the same importance in the activation of the rennin-zymogen of the juice as in the activation of the trypsinogen. This enzyme is also activated by enterokinase. The erepsin of the pancreatic juice (page 493) occurs as an active enzyme.

We are not quite clear whether the two other enzymes, the diastase and lipase, are secreted as such or as zymogens. It seems, nevertheless, that both are in part secreted as complete enzymes.

In the human embryo the trypsinogen and the erepsin (as well as also the pepsin) appear in the fourth and fifth fœtal month. The enterokinase appears at the same time or shortly after the trypsinogen.[5]

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[1] Bellamy, Journ. of Physiol., 27; Mendel and Rettger, Amer. Journ. of Physiol., 7. A very complete reference to the literature may be found in Menia Besbokaia Du rapport fonctionell entre le pankréas et la rate, Lausanne, 1901.

[2] Pflüger's Arch., 104., and 107.

[3] Vernon, Journ. of Physiol., 28; Hekma, Kon. Akad. v. Wetenschappen te Amsterdam, 1903, and Arch. f. (Anat. u) Physiol., 1904; Bayliss and Starling, Journ. of Physiol., 30

[4] Delezenne, Compt. rend. soc. biol., 59, 60, 62, 63; Zunz, footnote 1, p. 496.

[5] Ibrahim., Bioch. Zeitschr. 22, 24 (1909).

The way in which the trypsinogen is converted into trypsin is still unknown and is the subject of dispute. According to one view, proposed by PAWLOW and defended by BAYLISS and STARLING, the trypsinogen is transformed into trypsin by the action of the kinase. In the opinion of DELEZENNE, DASTRE, and STASSANO, and others,[1] the trypsin, on the contrary, is a combination of the kinase and trypsinogen, analogous to the cytotoxines, which, according to EHRLICH'S side-chain theory, are combinations between a complement and an amboceptor. (See page 69.)

The specific excitants for the secretion of pancreatic juice are, according to PAWLOW and his collaborators, acids of various kinds—hydrochloric acid as well as lactic acid—and fats, the latter acting probably by virtue of the soaps produced therefrom. Alkalies and alkali carbonates have, on the contrary, a retarding action. It appears that the acids act by irritating the mucosa of the duodenum. According to LONDON and SCHWARZ the secretion can also be excited from the entire jejunum and the upper part of the ileum. The secretion becomes weaker the further away the exciting source is from the duodenum.[2] Water, which causes a secretion of acid gastric juice, likewise becomes, indirectly, a stimulant for the pancreatic secretion, but may also be an independent exciter. The psychical moment may, at least in the first place, have an indirect action (secretion of acid gastric juice), and the food seems otherwise to have an action on pancreatic secretion by its action on the secretion of gastric juice.

The most important excitant for the secretion of juice is hydrochloric acid, but opinions are not in unison as to the manner in which the acid acts. PAWLOW'S school claims that the acid acts reflexly upon the intestine, causing a secretion of juice. That a reflex action is here produced is not contradicted by the investigations of POPIELSKI, WERTHEIMER and LEPAGE, FLEIG,[3] and others. According to the researches of BAYLISS and STARLING, which have been confirmed by CAMUS, GLEY, FLEIG, HERZEN, DELEZENNE, and others, a second factor must also be active here. BAYLISS and STARLING have shown that a body which they have called *secretin* can be extracted from the intestinal mucosa by a hydrochloric-acid solution of 4 p. m., and this when introduced into the blood produces a secretion of pancreatic juice, bile, and in the opinion of some investigators also of saliva and intestinal juice. The

[1] Bayliss and Starling, Journ. of Physiol., **30** and **32**, which also cities the other investigators and also O. Cohnheim, Bioch. Centralbl. **1**, 169 and S. Rosenberg, *ibid.*, **2**, 708.

[2] Zeitschr. f. physiol. Chem. **68**, 346 (1910) which also contains the literature.

[3] Fleig, Centralbl. f. Physiol., **16**, 681, and Compt. rend. soc. biol., **55**. See also footnote 1.

secretin, which according to BAYLISS and STARLING,[1] is the same in all vertebrates examined, is not destroyed by heat; it is therefore not identical with enterokinase, and is not considered an enzyme. It is formed from another substance, *prosecretin*, by the action of acids. According to DELEZENNE and POZERSKI secretin occurs as such in the intestinal mucosa, and the acids act only by the elimination of certain bodies having a retarding action. According to POPIELSKI secretin action is different from acid action; and the secretin action can also be obtained by WITTE'S peptone. He believes that the secretin is not a specific constituent of the intestine but a body widely distributed. GIZELT disputes the occurrence of a specific secretin and he compares this body to peptone. GLEY has obtained a solution which had a stronger secreting action than secretin by macerating the mucosa with proteoses.[2] v. FÜRTH and SCHWARZ[3] also call attention to the uncertainty of our knowledge as to the nature of secretin. According to them secretin is probably a mixture of bodies, among which probably the choline, found by them in the intestinal walls, acts the rôle of an exciter of secretion.

A second means of causing secretion is the fat, which probably only acts after it has been saponified. Oil-soap directly introduced into the duodenum brings about a strong secretion of pancreatic juice (SAWITSCH, BABKIN[4]), and at the same time a flow of bile, gastric juice, and the secretion of BRUNNER'S glands occurs. The pancreatic juice secreted under these circumstances has about the same amount of enzymes as the juice secreted after partaking of food.

We know very little as to how the soaps act. FLEIG[5] found that by maceration of the mucosa of the upper part of the duodenum with soap solution, a substance goes into solution which he calls *sapokrinin*, and which when introduced into the blood brings about a strong secretion of pancreatic juice. This sapokrinin, which is derived from a prosapokrinin. is not an enzyme and is not identical with secretin. After the action of chloral hydrate an abundant secretion occurs in the duodenum (WERTHEIMER and LEPAGE), which FALLOISE considers as produced by a special secretin, chloral secretin. The secretion of pancreatic juice can also be increased by alcohol, and FLEIG[6] claims to have obtained a secretin, *ethyl secretin*, by macerating the intestinal mucosa with alcohol. Further investigations are necessary of all these so-called secretins.

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[1] Journ. of Physiol., **29**.

[2] Delezenne and Pozerski, Compt. rend. soc. biol., **56**; Popielski, Centralbl. f. Physiol., **19**; Pflüger's Arch. **128**; Gizelt, Pflüger's Arch. 123; Gley, Compt. Rend. **151**, 345.

[3] v. Fürth and Schwarz, Pflüger's Arch. **124** (literature on secretin).

[4] Arch des scienc. biol. de St. Pétersbourg, **11**, and Zeitschr. f. physiol. Chem., **56**.

[5] Compt. rend. soc. biol., **55**, and Journ. de Physiol, et de Pathol. gén., 1904.

[6] Wertheimer and Lepage, Compt. rend. soc. biol., **52**; Fleig, *ibid.*, **55**; Falloise, Bull. Acad. Roy. Belg., 1903.

The estimation as to the quantity of pancreatic juice secreted in the twenty-four hours differs very much. According to the determinations of PAWLOW and his collaborators, KUWSCHINSKI, WASSILIEW, and JABLONSKY,[1] the average quantity (with normally acting juice) from a permanent fistula in dogs is 21.8 cc. per kilo in the twenty-four hours.

The pancreatic juice of the dog is a clear, colorless, and odorless alkaline fluid which when obtained from a temporary fistula is very rich in proteins, sometimes so rich that it coagulates like the white of the egg on heating. Besides *proteins*, the juice also contains the three above-mentioned enzymes (or their zymogens), *amylopsin*, perhaps also *maltase*, *trypsin*, *steapsin*, also an enzyme similar to erepsin, and besides these a *rennin*, which was first observed by KÜHNE. Besides the above-mentioned bodies the pancreatic juice invariably contains small quantities of *leucine*, *fat*, and *soaps*. As mineral constituents it contains chiefly alkali chlorides and considerable alkali carbonate, some phosphoric acid, lime, magnesia, and iron.

The quantity of solids in the pancreatic juice of the dog varies, as found by MAZURKIEWICZ, BABKINE and SAWITSCH,[2] according to the rapidity of secretion and the kind of excitant. As a rule the amount of solids is in inverse proportion to the rapidity of secretion. The juice secreted after the action of acids has the lowest amount of solids, 9–37.4 p. m. The juice after taking food is more concentrated, about 60–70 p. m. and that after vagus stimulation often contains 90 p. m. solids. The juice analyzed by C. SCHMIDT[3] from a temporary fistula contained 99–116 p. m. solids. The quantity of mineral bodies was 8.8 p. m.

The mineral constituents consisted chiefly of NaCl, 7.4 p. m., which is remarkable because the juice contains such a large amount of alkali carbonate. In the juice examined by DE ZILWA[4] the quantity of alkali in the secretin juice was 5–7.9 p. m. and in the pilocarpin juice 2.9–5.3 p. m. Na_2CO_3.

In the pancreatic juice of rabbits 11–26 p. m. solids have been found, and in that from sheep 14.3–36.9 p. m. In the pancreatic juice of the horse 9–15.5 p. m. solids have been found; in that of the pigeon, 12–14 p. m.

The human physiological pancreatic secretion from a fistula has been investigated by GLAESSNER.[5] The secretion was clear, foamed readily,

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[1] Arch. des sciences de St. Pétersbourg, **2**, 391. The previous claims of Bidder and Schmidt, and others may be found in Kühne, Lehrbuch, **114**.

[2] Mazurkiewicz, l. c.; Babkin and Sawitsch, Zeitschr. f. physiol. Chem., **56**.

[3] Cited from Maly in Hermann's Handbuch der Physiol., **5**, Theil II, 189.

[4] Journ. of Physiol., **31**.

[5] Zeitschr. f. physiol. Chem., **40**. See also Ellinger and Kohn, *ibid.*, **45**, and the investigations upon cystic fluids from the pancreas by Schumm, *ibid.*, **36**, and Murray and Gies, American Medicine, **4**, 1902; Glaessner and Popper, Deutsch. Arch. f. klin. Med. **94**, 46; see also Wohlgemuth, Bioch. Zeitschr. **39**; Bradley, Journ. of Biol. Chem. **6**.

had a strong alkaline reaction even toward phenolphthalein, and contained globulin and albumin but no proteoses and peptones. The specific gravity was 1.0075 and the freezing-point depression was $\Delta = -0.46$–$0.49°$. The solids were 12.44–12.71 p. m., the total protein 1.28–1.74 p. m., and the mineral bodies 5.66–6.98 p. m. The secretion contained trypsinogen, which was activated by the intestinal juice. Diastase and lipase were present; inverting enzymes, on the contrary, were not. The daily quantity of juice was 500–800 cc. The quantity of secretion, of ferments, and of alkalinity was lowest in starvation, but soon rose with the taking of food, and reached its maximum in about four hours.

Amylopsin, or **pancreatic diastase**, which, according to KOROWIN and ZWEIFEL, is not found in new-born infants and does not appear until more than one month after birth, seems, although not identical with ptyalin, to be closely related to it. Amylopsin acts very energetically upon boiled starch, and according to KÜHNE also upon unboiled starch, especially at 37 to 40° C., and according to VERNON[1] best at 35° C. It forms, similarly to the action of saliva, besides dextrin, chiefly isomaltose and maltose, with only very little glucose (MUSCULUS and v. MERING, KÜLZ and VOGEL[2]). The glucose is probably formed by the action of the invertin existing in the gland and juice. The pancreatic juice of the dog in fact, contains, according to BIERRY and TERROINE,[3] maltase, its action becomes apparent only after very faint acidification of the juice. According to RACHFORD the action of the amylopsin is not reduced by very small quantities of hydrochloric acid, but is diminished by larger amounts. VERNON, GRÜTZNER, and WACHSMANN find that the action is indeed accelerated by very small quantities of hydrochloric acid, 0.045 p. m., while alkalies in very small amounts have a retarding action. This retarding action of alkalies and hydrochloric acid may be stopped by bile (RACHFORD). WOHLGEMUTH as well as Minami[4] find that the action of diastase is increased to a high degree by bile. The active constituent of the bile was soluble in water and alcohol but was not identical with the bile salts or cholesterin. The statements in regard to the action of lecithin are contradictory.

Steapsin, or **Fat-splitting Enzyme.** The action of the pancreatic juice on fats is twofold. First, the neutral fats are split into fatty acids

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[1] Korowin, Maly's Jahresber., **3**; Zweifel, footnote 2, p. 456, Kühne, Lehrbuch, 117; Vernon, Journ. of Physiol., **27**.

[2] See footnote 5, p. 456.

[3] See Tebb. Journ. of Physiol., **15**; Bierry and Terroine, Compt. rend. soc. biolog., **58**; Bierry, *ibid.*, **62**.

[4] Rachford, Amer. Journ. of Physiol., **2**; Vernon. l. c.; Grützner, Pflüger's Arch. **91**; Wohlgemuth, Bioch. Zeitschr. **21**, 447 (1909); Minami, *ibid.*, **39**, 339 (1912).

and glycerin, which is an enzymotic process; and secondly, it has also the property of emulsifying the fats.

The action of the pancreatic juice in splitting the fats may be shown in the following way: Shake olive-oil with caustic soda and ether, siphon off the ether and filter if necessary, then shake the ether repeatedly with water and evaporate at a gentle heat. In this way is obtained a residue of fat free from fatty acids, which is neutral and which dissolves in acid-free alcohol and is not colored red by alkanet tincture. If such fat is mixed with perfectly fresh alkaline pancreatic juice or with a freshly prepared infusion of the fresh gland and treated with a little alkali or with a faintly alkaline glycerin extract of the fresh gland (9 parts glycerin and 1 part 1 per cent soda solution for each gram of the gland), and some litmus tincture added and the mixture warmed to 37° C., the alkaline reaction will gradually disappear and an acid one take its place. This acid reaction depends upon the conversion of the neutral fats by the enzyme into glycerin and free fatty acids. A very much used method consists in determining the acidity of the mixture by means of titration before and after the action of the juice or the infusion.

The action of the pancreatic juice in splitting fats is a process analogous to that of saponification, the neutral fats being decomposed, by the addition of the elements of water into fatty acids and glycerin according to the following equation. $C_3H_5.O_3.R_3$ (neutral fat)$+3H_2O=$ $C_3H_5.O_3.H_3$ (glycerin)$+3$(H.O.R) (fatty acid). This depends upon a hydrolytic splitting, which was first positively proved by BERNARD and BERTHELOT. The pancreas enzyme also decomposes other esters, just as it does the neutral fats (NENCKI, BAAS, LOEVENHART[1] and others). The fat-splitting action of the lipase is, according to PAWLOW, BRUNO and many others[2] aided in its action by the bile. ROSENHEIM and SHAW-MACKENZIE found that the lipase action was accelerated by hæmolytic substances, as well as by normal serum; this accelerating action was inhibited by cholesterin. The accelerating substance of the serum was dialyzable and resistant to heat. ROSENHEIM was able to divide the lipase existing in a glycerin extract of the pig pancreas into enzyme and co-enzyme (page 52); in diluting with water a precipitate occurred which contained the real thermolabile enzyme while the dialyzable, heat resisting co-enzyme remained in the

[1] Bernard, Ann. de chim. et physique (3), **25**; Berthelot, Jahresber, d. Chem., 1855, 733; Nencki, Arch. f. exp. Path. u. Pharm., **20**; Baas, Zeitschr. f. physiol. Chem., **14**, 416; Loevenhart, Journ. of Biol. Chem., **2**; Terroine and Z. Morel, Compt. rend. soc. biol., **65**, **66**.

[2] Bruno, Arch. des scienc. biol. de St. Pétersbourg, **7**; see also Loevenhart and Souder, Journ. of biol. Chem., **2**; v. Fürth and Schütz, Hofmeister's Beiträge, **9**; Terroine, Bioch. Zeitschr. **23**; Compt. rend. soc. biol. **68**, 439, 518, 666, 754 (1910).

filtrate.[1] In regard to the synthetic action of pancreatic lipase see page 60.

The fatty acids which are split off by the action of the pancreatic juice combine in the intestine with the alkalies, forming soaps, which have a strong emulsifying action on the fats, and thus the pancreatic juice becomes of great importance in the emulsification and the absorption of the fats.

Trypsin. The action of the pancreatic juice in digesting proteins was first observed by BERNARD, but first proved by CORVISART.[2] It depends upon a special enzyme called, by KÜHNE, trypsin. This enzyme as previously explained, does not occur in the gland as such, but as trypsinogen. According to ALBERTONI[3] this zymogen is found in the gland in the last third of the intra-uterine life. Enzymes more or less like trypsin occur in other organs, and are very widely diffused in the vegetable kingdom,[4] in yeast and in higher plants, and are also formed by various bacteria. The enzymes similar to trypsin occurring in the plant kingdom are, according to VINES, a mixture of *peptases*, which transform the proteins into peptone, and *ereptases*, which split the peptones into amino-acids.

As we know of so-called antienzymes for other enzymes, so we also have antitrypsins, and not only in the intestinal canal but also in the blood-serum (see page 63). The results as to the possibility of producing antitrypsins by immunization, is still disputed.

Trypsin, like other enzymes, has not been prepared in a pure condition. Nothing is positively known in regard to its nature, but as obtained thus far it shows a variable behavior (KÜHNE, KLUG, LEVENE, MAYS, and others). At least it does not seem to be a nucleoprotein, and trypsin has also been obtained which did not give the biuret test (KLUG, MAYS, SCHWARZSCHILD). Trypsin dissolves in water and glycerin, while KÜHNE's trypsin was insoluble in glycerin. It is very sensitive to heat, and even the body temperature gradually decomposes it (VERNON, MAYS). In neutral solution it becomes inactive at 45° C. In dilute soda solution of 3–5 p. m. it is still more readily destroyed (BIERNACKI, VERNON[5]).

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[1] Journ. of Physiol. **40** (1910).

[2] Gaz. hebdomadaire, 1857, Nos. 15, 16, 19, cited from Bunge, Lehrbuch, 4, Aufl., 185.

[3] See Maly's Jahresber., 8, 254.

[4] In this connection see Vines, Annals of Botany, **16**, **17**, **18**, **19**, **22**, and **23**, and Oppenheimer, Die Fermente, 1910.

[5] Kühne, Verh. d. naturh.-med. Vereins zu Heidelberg (N. F.), **1**, 3; Klug, Math. naturw. Ber. aus Ungarn., **18**, 1902; Levene, Amer. Journ. of Physiol., **5**; Mays, Zeitschr. f. Physiol. Chem., **38**; Vernon, Journ. of Physiol., **28** and **29**; Biernacki, Zeitschr. f. Biologie, **28**; Schwarzschild, Hofmeister's Beiträge, **4**.

The presence of protein or proteoses has, to a certain extent, a protective action on heating an alkaline trypsin solution, and this has been substantiated by recent investigations of BAYLISS and VERNON. The simpler cleavage products have a still greater protective action (VERNON [1]). Trypsinogen, according to the unanimous statements of several experimenters, is more resistant toward alkalies than trypsin. Trypsin is gradually destroyed by gastric juice and even by digestive hydrochloric acid alone.

The preparation of pure trypsin has been tried by various experimenters. The most careful work in this direction was done by KÜHNE and MAYS. Various methods have been suggested by MAYS, but we cannot enter into a discussion of them. A very pure preparation can be obtained by making use of the combined salting out with NaCl and $MgSO_4$. A very active solution, and one that can be kept for a long time (for more than twenty years according to HAMMARSTEN), can be obtained by extracting with glycerin (HEIDENHAIN [2]). An impure but still very active infusion can be obtained after a few days by allowing the finely divided gland to stand with water which contains 5–10 cc. chloroform per liter (SALKOWSKI) at the temperature of the room. Such infusions can be obtained, nearly free from proteins, by dialyzing with running water after the addition of toluene.

Like other enzymes, trypsin is characterized by its action, and this action consists in dissolving protein and in splitting it into simpler products, mono- and diamino-acids, tryptophane, etc., in alkaline, neutral, and indeed in very faintly acid solutions. This action has been so far considered as characteristic for trypsin. Recent investigations seem to indicate that this action is not due to one enzyme alone, but to the combined action of several enzymes.

Although contrary to MAY'S statement, there is no question that there occurs in the pancreas besides trypsin, an enzyme similar to erepsin (BAYLISS and STARLING, VERNON [3]). According to the latter this erepsin has a strong action upon peptone, and he believes that the peptone-splitting action of a pancreas infusion is in great part due to the erepsin. The pancreas, besides these, also contains a *nuclease* (see page 493), whose relation to pancreas erepsin has not been determined.

The unity of trypsin has also been disputed from another point of view. According to POLLAK the trypsin (in the ordinary sense) contains a second enzyme, which does not act upon protein, but only upon gelatin, and he calls

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[1] Bayliss, Arch. des scienc. biolog. de St. Pétersbourg. **11**, Suppl.; Vernon, Journ. of Physiol., **31**.

[2] Pflüger's Arch., **10**.

[3] Bayliss and Starling, Journ. of Physiol., **30**; Vernon, *ibid.*, **30**; and Zeitschr. f. physiol. Chem., **50**; Mays, *ibid.*, **49** and **51**.

this enzyme *glutinase*. This glutinase is much more resistant toward acids than trypsin, and by proper treatment with acids POLLAK was able to change a pancreas infusion so that it acted upon gelatin and not upon certain proteins. The correctness of these observations has, indeed, not been generally accepted, and it is disputed by ASCOLI and NEPPI.[1] According to them the action of the trypsin is weakened by the acid, and indeed to such varying degrees for different proteins that the action upon albumin is lost while the action upon gelatin is noticeable. Nevertheless, we here have a warning to be careful as to the conclusions drawn from results where impure infusions are used. For many experiments it is undoubtedly advisable to use the natural pancreatic juice.

The following reports on the action of trypsin applies to the so-called trypsin, with the reservation that it is perhaps not a unit enzyme.

The *action of trypsin on proteins* is best demonstrated by the use of fibrin. Very considerable quantities of this protein body are dissolved by a small amount of trypsin at 37–40° C. It is always necessary to make a control test with fibrin alone, with or without the addition of alkali. Fibrin is dissolved by trypsin without any putrefaction; the liquid has a pleasant odor somewhat like bouillon. To completely exclude putrefaction a little thymol, chloroform, or toluene should be added to the liquid. Tryptic digestion differs essentially from peptic digestion, irrespective of the difference in the digestive products, in that the first takes place in neutral or alkaline reaction and not, as is necessary for peptic digestion, in an acidity of 1–2 p. m. HCl, and further by the fact that the proteins dissolve in trypsin digestion without previously swelling up.

As trypsin not only dissolves proteids, but also other protein substances such as gelatin, this latter body may be used in detecting trypsin. The liquefaction of strongly disinfected gelatin is, according to FERMI,[2] a very delicate test for trypsin or tryptic enzymes. Various suggestions for the use of gelatin in the trypsin test have been made. In consideration of the observations of ASCOLI and NEPPI that a trypsin may not act upon fibrin or other proteids but still digest gelatin, it is advisable never to make use of gelatin or proteid alone in testing for trypsin, but always the two.

For the quantitative estimation of trypsin by measuring the rapidity of digestion we generally make use of the method of METT, described under pepsin digestion. Another method, suggested by WEISS, consists in determining the nitrogen in the filtrate after coagulation with heat and acetic acid. LÖHLEIN recommends the titration method of VOLHARD as used in pepsin determinations, and has given directions for its use. JACOBY recommends the use of ricin, and GROSS suggests a method based upon the precipitation of casein by acid. BAY-

[1] Pollak, Hofmeister's Beiträge, 6; contradictory statements are found in Ehrenreich, cited in Bioch. Centralbl., 4; Ascoli and Neppi, Zeitschr. f. physiol. Chem., 56.

[2] Arch. f. Hyg. 12 and 55.

LISS follows the digestion by the electrical conductivity, and F. WEISS [1] determines the quantity of nitrogen not precipitated by tannic acid. The formol titration can also be used with advantage for determining the decomposition (page 166).

The reaction has a great influence upon the *rapidity of the trypsin digestion*. Trypsin acts energetically in neutral, or still better in alkaline, solutions, and according to older statements, best in an alkalinity of 3–4 p. m. Na_2CO_3; but the nature of the protein is also of importance. The reports in regard to the action of trypsin in various reactions are still somewhat disputed.[2] The action of the alkali depends upon the number of hydroxyl ions (DIETZE, KANITZ), and according to KANITZ [3] the digestion proceeds best in those solutions which are 1/70–1/200 normal in regard to hydroxyl ions. Free mineral acids, even in very small quantities, completely prevent the digestion. If the acid is not actually free, but combined with protein bodies, then the digestion may take place quickly when the acid combination is not in too great excess (CHITTENDEN and CUMMINS). Organic acids act less disturbingly, and in the presence of 0.2 p. m. lactic acid and the simultaneous presence of bile and common salt, the digestion may indeed proceed more quickly than in a faintly alkaline liquid (LINDBERGER). The assertion of RACHFORD and SOUTHGATE, that the bile can prevent the injurious action of the hydrochloric acid, and that a mixture of pancreatic juice, bile, and hydrochloric acid digests better than a neutral pancreatic juice, could not be substantiated by CHITTENDEN and ALBRO. That bile has an action tending to aid the tryptic digestion has been shown by many investigators, and recently by BRUNO, ZUNTZ and Ussow and others.[4] Carbon dioxide, according to SCHIERBECK,[5] has a retarding action in acid solutions, but it accelerates the tryptic digestion in faintly alkaline liquids. *Foreign bodies*, such as potassium cyanide, may promote tryptic digestion, while other bodies, such as salts of mercury, iron, and others (CHITTENDEN and CUMMINS), or salicylic acid in large quantities, may have a preventive action. According to WEISS [6] the halogen

[1] Weiss, Zeitschr. f. physiol. Chem., 40; Löhlein, Hofmeister's Beiträge, 7; Jacoby, Bioch. Zeitschr., 10; Gross, Arch. f. exp. Path. u. Pharm., 58; Bayliss, Arch. des scienc. biol. de St. Pétersbourg, 11, Suppl.; and Journ. of Physiol., 36; Weiss, Zeitschr. f. physiol. Chem. 31, 78 (1900).

[2] See Kudo, Bioch. Zeitschr., 15.

[3] Kanitz, Zeitschr. f. physiol. Chem., 37, who also cites Dietze.

[4] Chittenden and Cummins, Studies from the Physiol. Chem. Laboratory of Yale College, New Haven, 1885, 1, 100; Lindberger, Maly's Jahresber., 13; Rachford and Southgate, Medical Record, 1895; Chittenden and Albro, Amer. Journ. of Physiol., 1, 1898; Rachford, Journ. of Physiol., 25; Bruno, l. c.; Zuntz and Ussow, Arch. f. (Anat. u.) Physiol., 1900.

[5] Skand. Arch. f. Physiol., 8.

[6] Weiss, Zeitschr. f. physiol. Chem., 40; See also Kudo, Bioch. Zeitschr. 15, 473 (1908).

alkali salts disturb tryptic digestion only slightly, and NaCl seems to have the strongest action. The sulphates have a much stronger retarding action than the chlorides. The *nature of the proteins* is also of importance. Unboiled fibrin is, relatively to most other proteins, dissolved so very quickly that the digestion test with raw fibrin gives an incorrect idea of the power of trypsin to dissolve coagulated protein bodies in general. Boiled fibrin is digested with much greater difficulty and also requires a higher alkalinity: 8 p. m. Na_2CO_3 (VERNON [1]). The resistance of certain native protein solutions, such as blood-serum and egg-white, against the action of trypsin is remarkable. In regard to the inhibition of the action of trypsin see Chapter I, page 63.

The Products of the Tryptic Digestion. In the digestion of unboiled fibrin a globulin which coagulates at 55–60° C. may be obtained as an intermediary product (HERRMANN [2]). Besides this, one obtains from fibrin, as well as from other proteins, the products previously mentioned in Chapter II. In trypsin digestion the cleavage may proceed so far that the mixture fails to give the biuret reaction. This does not indicate, as E. FISCHER and ABDERHALDEN have shown, a complete cleavage of the protein molecule into mono- and diamino-acids, etc. In tryptic digestion, as shown by ABDERHALDEN and REINBOLD, using the protein edestin, and by ABDERHALDEN and VOEGTLIN [3] with casein, a gradual cleavage of the protein takes place, and thereby certain amino-acids, like tyrosine and tryptophane, are readily and completely split off, while others, like leucine, alanine, aspartic acid, and glutamic acid, are slowly and less readily split off, and others, such as α-proline, phenylalanine, and glycocoll, stubbornly resist the cleavage action of the trypsin. The polypeptide-like bodies discovered by FISCHER and ABDERHALDEN, which are produced in digestion, and which do not give the biuret reaction, are the atomic complexes which resist the action of trypsin. These peptoids contain the pyrrolidine carboxylic acid and phenylalanine groups of the protein, but also yield other monamino-acids such as leucine, alanine, glutamic acid, and aspartic acid. Among the above-mentioned products we find on the autodigestion of the gland other substances, such as oxyphenylethylamine (EMERSON), which is produced from tyrosine by fermentive CO_2 cleavage, also uracil (LEVENE), guanidine (KUTSCHER and OTORI), the purine bases, which originate from the nuclein bodies, and choline, which latter is formed from lecithin

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[1] Journ. of Physiol., 28.

[2] Hermann, Zeitschr. *f.* physiol. Chem., 11.

[3] Abderhalden and Reinbold, Zeitschr. f. physiol. Chem., 44 and 46, with Voegtlin, *ibid.* 53.

(KUTSCHER and LOHMANN [1]). If putrefaction is not completely prevented, still other bodies occur which will be considered later in connection with the putrefactive processes in the intestine.

The Action of Trypsin upon other Bodies. The *nucleoproteins* and *nucleins* are so digested that the protein complex is separated from the nucleic acid and then digested. The nucleic acids may, nevertheless, be somewhat changed (ARAKI), which is probably brought about by another enzyme, the *nuclease* (SACHS). A cleavage of nucleic acids with the setting free of phosphoric acid and purine bases is, according to IWANOFF,[2] not brought about by trypsin. The splitting is first produced by the action of nuclease or erepsin (see page 493). *Gelatin* is dissolved and digested by pancreatic juice. A cleavage with the separation of glycocoll and leucine does not occur (KÜHNE and EWALD), or only to a trivial extent (REICH-HERZBERGE [3]).

The *gelatin-forming substance* of the connective tissues is not directly dissolved by trypsin, but only after it has been treated with acids or soaked in water at 70° C. By the action of trypsin on hyaline *cartilage* the cells dissolve, leaving the nucleus. The matrix is softened and shows an indistinctly constructed network of collagenous substances (KÜHNE and EWALD). The *elastic substance*, the *structureless membranes*, and the *membrane of the fat-cells*, are also dissolved. *Parenchymatous organs*, such as the liver and the muscles, are dissolved all but the nuclei, connective tissue, fat-corpuscles, and the remainder of the nervous tissue. If the muscles are boiled, then the connective tissue is also dissolved. *Mucin* is dissolved and split by trypsin, while *chitin* and *horn substance* do not seem to be acted upon by the enzyme. *Oxyhæmoglobin* is decomposed by trypsin with the splitting off of hæmatin. Trypsin splits off large amounts of hydriodic acid from diiodotyrosine (OSWALD [4]). Trypsin has no action upon fats and carbohydrates.

The action of trypsin on simply constructed substances of known constitution such as acid-amides, polypeptides, is of especially great interest. In this regard we have the somewhat earlier investigations of GULEWITSCH, GONNERMANN, and SCHWARZSCHILD,[5] but the investi-

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[1] Fischer and Abderhalden, Zeitschr. f. physiol. Chem., **39**; Emerson, Hofmeister's Beiträge, **1**; Levene, Zeitschr. f. physiol. Chem., **37**; Kutscher and Lohmann, *ibid.* **39**; Kutscher and Otori, *ibid.*, **43**, and Centralbl. f. Physiol., **18**.

[2] Iwanoff, Zeitschr. f. physiol. Chem., **39**, which also contains the literature; Sachs, *ibid.*, **46**.

[3] Kühne and Ewald, Verh. d. naturh.-med. Vereins zu Heidelberg (N. F.), **1**; Reich-Herzberge, Zeitschr. f. physiol. Chem., **34**.

[4] Zeitschr. f. physiol. Chem. **62**, 432 (1909).

[5] Hofmeister's Beiträge, **4**, where the other works are also cited.

gations of FISCHER and of ABDERHALDEN and their co-workers,[1] are much more complete and important. In this connection see page 62.

The behavior of the polypeptides with trypsin, or closely related enzymes, is of the greatest interest and in many respects very important. Thus in the polypeptides we have a means of determining the kind of enzyme, whether it belongs to the pepsin, trypsin, or erepsin group. We know of no polypeptide which is split by pepsin; trypsin splits only certain polypeptides, but not others, while the erepsin on the contrary seems to split all polypeptides, occurring in nature, which are composed of aminoacids. By the aid of the polypeptide reaction ABDERHALDEN and co-workers have also been able to show that the trypsin-like enzyme, occurring in the blood-plasma, is not identical with trypsin because it does not attack glycyl-*l*-tyrosine, which is split by trypsin.

Pancreatic rennin is an enzyme found in the gland and in the juice, which coagulates neutral or alkaline milk (KÜHNE and ROBERTS and others). This enzyme, according to PAWLOW'S school, is identical with trypsin. The similarity of action of these two enzymes and the fact that both are activated simultaneously from the zymogens by enterokinase or lime salts (DELEZENNE, WOHLGEMUTH [2]) seem to point to this identity. On the other hand the optimum of the enzyme action for the pancreatic rennin is 60–65° C. (VERNON), which is much higher than for the trypsin, and GLAESSNER and POPPER [3] have also observed a case where the human pancreatic juice contained no rennin enzyme.

According to HALLIBURTON and BRODIE,[4] casein is converted by the pancreatic juice of the dog into "pancreatic casein," a substance which, in regard to solubility, stands to a certain extent between casein and paracasein (see Chapter XIII), and which is converted into paracasein by rennin. Further investigations on the action of this enzyme upon milk and especially upon pure casein solutions are very desirable.

Pancreatic Calculi. The concrement from a cystic enlargement of WIRSUNG'S duct in a man, as analyzed by BALDONI, contained in 1000 parts as follows: Water 34.4, ash 126.7, protein substances 34.9, free fatty acids 133, neutral fats 124, cholesterin 70.9, soaps and pigment 499.1, parts. SCHEUNERT and BERGHOLZ [5] have reported a pancreatic calculi in the ox.

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[1] Fischer and Bergell, Ber. d. d. chem. Gesellsch., **36** and **37**; Fischer and Abderhalden, Sitzungsber. der Kgl. Pr. Akad. d. Wissensch., Berlin, 1905. The works of Abderhalden and co-workers cannot be specially cited, but may be found in Zeitschr. f. physiol. Chem., **47**, **48**, **49**, **51**, **52**, **53**, **54**, **55**, and **57**.

[2] Kühne and Roberts, Maly's Jahresber., **9**; see also Edkins, Journ. of Physiol., **12** (literature); Delezenne, Compt. rend. soc. biol., **62** and **63**; Wohlgemuth, Bioch. Zeitschr., **2**.

[3] Vernon, Journ. of Physiol., **12**; Glaessner and Popper, Deutsch. Arch. f. klin. Med., **94**.

[4] Journ. of Physiol., **20**.

[5] Baldoni, Maly's Jahresb., **29**, 353; Scheunert and Bergholz., Zeitschr. f. physiol. Chem., **52**.

Besides the enzymes which have been discussed in connection with the pancreatic juice, the gland also contains others, among which can be mentioned the enzyme which, according to STOKLASA and his collaborators, occurs principally in organs and tissues and which decomposes sugar into alcohol and carbon dioxide, like zymase. Opinions as to the importance of the pancreas for glycolysis are diverse, and we therefore refer the reader to what has been previously stated on this subject in Chapter VII, pages 407 and 408.

V. THE CHEMICAL PROCESSES IN THE INTESTINE.

The action which belongs to each digestive secretion may be essentially changed under certain conditions by being mixed with other digestive fluids for various reasons, and also by the action of the enzymes upon each other;[1] and since the digestive fluids which flow into the intestine are mixed with still another fluid, the bile, it will be readily understood that the combined action of all these fluids in the intestine makes the chemical processes going on therein very complicated.

As the acid of the gastric juice acts destructively on ptyalin, this enzyme has no further diastatic action, even after the acid of the gastric juice has been neutralized in the intestine. ROGER and SIMON[2] claim to have observed in saliva made inactive by the gastric juice, a reactivation caused by the pancreatic juice, but these investigations do not seem to be fully conclusive. The bile has, at least in certain animals, a slight diastatic action, which in itself can hardly be of any great importance, but which shows that the bile has not a preventive, but rather a beneficial influence on the energetic diastatic action of the pancreatic juice. Several experimenters[3] have observed a beneficial action of the bile on the diastatic action of the pancreas infusion. To this may be added that the micro-organisms which habitually occur in the intestine and sometimes in the food have partly a diastatic action and partly produce a lactic-acid and butyric-acid fermentation. The maltose which is formed from the starch seems to be converted into glucose in the intestine. It seems conclusively that the cellulose cannot be digested in the organism of the dog.[4] LOHRISCH found that on an average of 50 per cent of the introduced cellulose and hemicellulose was digested in human beings and yielded the corresponding sugar. That

[1] See Wróblewski and collaborators, Hofmeister's Beiträge, 1.

[2] Compt. rend. soc. biol., 62.

[3] Martin and Williams, Proceed. of Roy. Soc., 45 and 48; Bruno, footnote 2, p. 502; Buglia, Bioch. Zeitschr. 25.

[4] Scheunert, cited from Bioch. Centralbl. 10, 71; see also Lorhisch, Zeitschr. f. physiol. Chem. 69, 143 (1910) as well as Bioch. Centralbl. 8, 334.

cellulose undergoes a fermentation in the intestine by the action of micro-organisms, producing marsh-gas, acetic acid, and butyric acid, has been especially shown by TAPPEINER; still it is not known to what extent the cellulose is destroyed in this way.[1]

The bile has, as shown by MOORE and ROCKWOOD[2] and then especially by PFLÜGER, the property to a high degree of dissolving fatty acids, especially oleic acid, which itself is a solvent for other fatty acids, and hence, as will be seen later, it is of great importance in the absorption of fat. It is also of great importance that the bile, as previously stated, not only activates the steapsinogen, but that, as first shown by NENCKI and RACHFORD,[3] it accelerates the fat-splitting action of the steapsin. According to v. FÜRTH and SCHÜTZ[4] the bile-salts are the active constituents of the bile in this cleavage, and the fatty acids set free can combine with the alkalies of the intestinal and pancreatic juices and the bile, producing soaps which are of great importance in the emulsification of the fats.

If to a soda solution of about 1–3 p. m. pure, perfectly neutral olive-oil is added in not too large a quantity, a transient emulsion is obtained after vigorous shaking. If, on the contrary, one adds to the same quantity of soda solution an equal amount of commercial olive-oil (which always contains free fatty acids), the vessel need only be turned over for the two liquids to mix, and immediately there appears a very finely divided and permanent emulsion, making the liquid appear like milk. The free fatty acids of the commercial oil, which is always somewhat rancid, combine with the alkali to form soaps which act to emulsify the fats (BRÜCKE, GAD, LOEWENTHAL[5]). This emulsifying action of the fatty acids split off by the pancreatic juice is undoubtedly assisted by the habitual occurrence of free fatty acids in the food, as well as by the splitting off of fatty acids from the neutral fats in the stomach (see page 476).

Bile completely prevents peptic zymolysis in artificial digestion,

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[1] On the digestion of cellulose see Henneberg and Stohmann, Zeitschr, f. Biologie, **21**, 613; v. Knieriem, *ibid.*, 67; Hofmeister, Arch. f. wiss. u. prakt. Thierheilkunde, **11**; Weiske, Zeitschr. f. Biologie, **22**, 373; Tappeiner, *ibid.*, **20** and **24**; Mallèvre, Pflüger's Arch., **49**; Omeliansky, Arch. d. scienc. biol. de St. Pétersbourg, **7**; E. Müller, Pflüger's Arch., **83**; Lohrisch, Zeitschr. f. physiol. Chem., **47** (literature); Pringsheim, *ibid.* **78**, 266 (1912).

[2] Proceedings of Roy. Soc., **60**, and Journ. of Physiol., **21**. In regard to Pflüger's work see Absorption.

[3] Nencki, Arch. f. exp. Path. u. Pharm., **20**; Rachford, Journal of Physiol., **12**.

[4] Centralbl. f. Physiol., **20**.

[5] Brücke, Wien, Sitzungsber., **61**, Abt. 2; Gad, Arch. f. (Anat. u.) Physiol., 1878; Loewenthal, *ibid.*, 1897.

because it retards the swelling up of the proteins. The passage of bile into the stomach during digestion on the contrary, seems, according, to several investigators, especially ODDI and DASTRE,[1] to have no disturbing action on gastric digestion. According to BOLDYREFF,[2] after continuous starvation, on feeding fat and food rich in fat, as well as after large amounts of acid, a mixture of bile, pancreatic juice, and intestinal juice pass readily into the stomach. After food rich in fat, which retards the secretion of gastric juice and the motility of the stomach, a digestion due to this alkaline mixture may take place in the stomach.

Bile itself has no solvent action on proteins in neutral or alkaline reaction, but still it may exert an influence on protein digestion in the intestine. The acid contents of the stomach, containing an abundance of proteins, give with the bile a precipitate of proteins and bile-acids. This precipitate carries a part of the pepsin with it, and for this reason, and also on account of the partial or complete neutralization of the acid of the gastric juice by the alkali of the bile and the pancreatic juice, the pepsin digestion cannot proceed further in the intestine. According to BAUMSTARK and COHNHEIM[3] connective tissue is digested on the other side of the pylorus in the intestine by the pepsin-hydrochloric acid. On the contrary, the bile does not disturb the digestion of proteins by the pancreatic juice in the intestine. The action of these digestive secretions, as above stated, is not disturbed by the bile, not even by the faintly acid reaction due to organic acids; but, on the contrary, the action of trypsin is accelerated by the bile. In a dog killed while digestion is going on, the faintly acid, bile-containing material of the intestine shows regularly a strong digestive action on proteins.

The precipitate of protein and bile-salts formed on the meeting of the acid contents of the stomach with the bile easily redissolves in an excess of bile, and also in the NaCl formed in the neutralization of the hydrochloric acid of the gastric juice. This may take place even in faintly acid reaction. Since in man the excretory ducts of the bile and the pancreatic juice open near one another, in consequence of which the acid contents of the stomach are probably immediately in great part neutralized by the bile as soon as it enters, it is doubtful whether a precipitation of proteins by the bile occurs in the intestine.

Besides the previously mentioned processes caused by enzymes, there are others of a different nature going on in the intestine, namely, the fermentation and putrefaction processes caused by micro-organisms. These are less intense in the upper parts of the intestine, but

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[1] Oddi, in Centralbl. f. Physiol., **1**, 312; Dastre, Arch. de Physiol. (5), **2**, 316.

[2] Centralbl. f. Physiol., **18**, 457, and Pflüger's Arch., **121**.

[3] Zeitschr. f. physiol. Chem., **65**, 477 (1910).

increase in intensity toward the lower part, and decrease in the large intestine because of the consumption of fermentable material and by the removal of water by absorption. Fermentation processes, but only very slight putrefaction, occur in the small intestine of man. MACFADYEN, M. NENCKI, and N. SEEBER[1] have investigated a case of human anus præternaturlis, in which the fistula occurred at the lower end of the ileum, and they were able to investigate the contents of the intestine after it had been exposed to the action of the mucous membrane of the entire small intestine. The mass was yellow or yellowish-brown, due to bilirubin, and had an acid reaction which, on a mixed but principally animal diet, calculated as acetic acid, amounted to 1 p. m. The contents were nearly odorless, having an empyreumatic odor recalling that of volatile fatty acids, and infrequently had a putrid odor resembling that of indol. The essential acid present was acetic acid, accompanied by fermentation and paralactic acid, volatile fatty acids, succinic acid, and bile-acids. Coagulable proteins, peptone, mucin, dextrin, sugar, and alcohol were present. Leucine and tyrosine could not be detected.

According to the above-mentioned investigators, the proteins are only to a very slight extent, if at all, decomposed by the microbes in the small intestine of man. The organisms present in the small intestine preferably decompose the carbohydrates, forming ethyl alcohol and the above-mentioned organic acids.

Further investigations of JAKOWSKY and of AD. SCHMIDT[2] lead to the same result, namely, that in man the putrefaction of the proteins takes place chiefly in the large intestine, and the conditions are the same in carnivora. In these latter it has been possible to follow the intestinal digestion by investigating the contents of the various parts of the intestine as well as by forming fistulas along the intestine. Again PAWLOW and his pupils, especially LONDON[3] and his collaborators, have essentially advanced our knowledge on this subject.

In regard to the digestion of protein, it has been found that after feeding meat, bread, or certain protein bodies, the digestion in the stomach and small intestine is so complete that on the passage of the contents into the cæcum all the protein is digested and absorbed. Unboiled white of egg is an exception and is digested with difficulty. In experiments with unboiled white of egg, LONDON and SULEIMA reob-

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[1] Arch. f. exp. Path. u. Pharm., 28.

[2] Jakowsky, Arch. des scienc. biol. de St. Pétersbourg, 1; Ad. Schmidt, Arch. f. Verdauunskr., 4.

[3] The works of London and collaborators cannot be cited in detail, but may be found in Zeitschr. f. physiol. Chem., 46–57.

tained 73 per cent of the coagulable protein from a fistula in the ileum (2–3 cm. in front of the cæcum). Elastin is, according to LONDON,[1] more slowly digested in the small intestine than other proteins. KUTSCHER and SEEMANN, ABDERHALDEN, LONDON and collaborators[2] have also found that non-biuret giving products and amino-acids are regularly split off, probably by the combined action of trypsin and erepsin. These amino-acids occur to a slight extent only, but from this no conclusion can be drawn as to the extent of amino-acid formation, because we do not know the extent of their absorption. The digestion of protein in the intestine, it seems, according to ABDERHALDEN, LONDON, OPPLER and REEMLIN,[3] is similar to the artificial digestion with trypsin, namely, that the destruction takes place step-wise, that certain amino-acids, such as tyrosine, are split off earlier than others. ZUNZ[4] found the same end result in the protein cleavage in the small intestine, with bread as with meat feeding. LONDON, SCHITTENHELM and WIENER[5] found that a cleavage of nucleic acids with the formation of nucleosides occurred in the lower part of the jejunum and ileum.

The decomposition products of the proteins formed by the action of gastric juice can, according to LONDON,[6] be absorbed without further cleavage by the pancreatic juice, and a further cleavage in the intestine seems to be more necessary for assimilation than for absorption.

The carbohydrates and the fats (LEVITES[7]) may be so completely split in the stomach and small intestine that their absorption is complete before the contents pass into the cæcum. According to LONDON and POLOWZOWA[8] a strong cleavage of starch, dextrins and disaccharides takes place, especially in the duodenum, while the absorption is less strong here. The carbohydrates are here prepared for the absorption taking place in the lower parts of the intestine, though the cleavage also goes on in the other parts, namely in the jejunum and the upper part of the ileum.

As above remarked, ordinarily no putrefaction takes place in the small intestine, but occurs generally only in the large intestine. This

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[1] London and Suleima, Zeitschr. f. physiol. Chem., **46**; London, *ibid.*, **60**.

[2] Kutscher and Seemann, *ibid.*, **34**; Abderhalden and London, with Kautzsch, *ibid.*, with L. Baumann, *ibid.*, **51**, with v. Körösy, *ibid.*, **53**.

[3] Zeitschr. f. physiol. Chem., **55** and **58**.

[4] Intern. Beitr. z. Pathol. u. Ther. d. Ernährungsstörungen, **2**, 195, 459 (1910 and 1911).

[5] Zeitschr. f. physiol. Chem., **72**, 459 (1911).

[6] *Ibid.*, **49**.

[7] *Ibid.*, **49** and **53**.

[8] *Ibid.*, **56**.

putrefaction of the proteins is not the same as the pancreatic digestion. In putrefaction the decomposition goes much further, and a mixture of products is obtained which have become known through the labors of numerous investigators, especially NENCKI, BAUMANN, BRIEGER, H. and E. SALKOWSKI, and their pupils. The products which are formed in the putrefaction of proteins are (in addition to *proteoses*, *peptones*, *amino-acids*, and *ammonia*) *indol*, *skatol*, *paracresol*, *phenol*, *phenylpropionic acid*, and *phenylacetic acid*, also *paraoxyphenylacetic acid* and *hydroparacoumaric acid* (besides paracresol, produced in the putrefaction of tyrosin), *volatile fatty acids*, *carbon dioxide*, *hydrogen*, *marsh-gas*, *methylmercaptan*, and *sulphureted hydrogen*. In the putrefaction of gelatin neither tyrosine nor indol is formed, while *glycocoll* is produced instead.

Among these products of decomposition a few are of special interest because of their behavior within the organism and because after their absorption they pass into the urine. A few, such as the oxyacids, pass unchanged into the urine. Others, such as phenols, are directly transformed into ethereal sulphuric acids by synthesis, and are eliminated as such by the urine; on the contrary, others, such as indol and skatol, are converted into ethereal sulphuric acids after oxidation only (for details see Chapter XIV). The quantity of these bodies in the urine also varies with the extent of the putrefactive processes in the intestine; at least this is true for the ethereal sulphuric acids. Their quantity increases in the urine with a stronger putrefaction, and the reverse takes place, namely, a disappearance from the urine, or a great reduction in quantity, as BAUMANN, HARLEY and GOODBODY[1] have shown by experiments on dogs, when the intestine is disinfected by various agents.

The *gases* which are produced by the decomposition processes are mixed in the intestinal tract with the atmospheric air swallowed with the saliva and food, and as the gas developed in the decomposition of different foods varies, so the mixture of gases after various foods should have a dissimilar composition. This is found to be true. *Oxygen* is found only in very faint traces in the intestine; this may be accounted for in part by the formation of reducing substances in the fermentation processes which combine with the oxygen, and partly, perhaps chiefly, to a diffusion of the oxygen through the tissues of the walls of the intestine. To show that these processes take place mainly in the stomach, the reader is referred to page 486, on the composition of the gases of the stomach. *Nitrogen* is invariably found in the intestine, and it is probably due chiefly to the swallowed air. The *carbon dioxide*

[1] Baumann, Zeitschr. f. physiol. Chem., 10; Harley and Goodbody, Brit. Med. Journ., 1899.

originates partly from the contents of the stomach, partly from the putrefaction of the proteins, partly from the lactic-acid and butyric-acid fermentation of carbohydrates, and partly from the setting free of carbon dioxide from the alkali carbonates of the pancreatic and intestinal juices by their neutralization through the hydrochloric acid of the gastric juice and by organic acids formed in the fermentation. *Hydrogen* occurs in largest quantities after a milk diet, and in smallest quantities after a purely meat diet. This gas seems to be formed chiefly in the butyric-acid fermentation of carbohydrates, although it may occur in large quantities in the putrefaction of proteins under certain circumstances. There is no doubt that the *methylmercaptan* and *sulphureted hydrogen* which occur normally in the intestine originate from the proteins. The *marsh-gas* undoubtedly originates in the putrefaction of proteins. As proof of this RUGE [1] found 26.45 per cent marsh-gas in the human intestine after a meat diet. He found a still greater quantity of this gas after a vegetable (leguminous) diet; this coincides with the observation that marsh-gas may be produced by a fermentation of carbohydrates, but especially of cellulose (TAPPEINER [2]). Such an origin of marsh-gas, especially in herbivora, is to be expected. A small part of the marsh-gas and carbon dioxide may also arise from the decomposition of lecithin (HASEBROEK [3]).

Putrefaction in the intestine not only depends upon the composition of the food, but also upon the albuminous secretions and the bile. Among the constituents of bile which are changed or decomposed, there are not only the pigments—the bilirubin yields urobilin and a brown pigment—but also the bile-acids, especially taurocholic acid. Glycocholic acid is more stable, and a part is found unchanged in the excrement of certain animals, while taurocholic acid is so completely decomposed that it is entirely absent in the feces. In the fetus, on the contrary, in whose intestinal tract no putrefaction processes occur, undecomposed bile-acids and bile-pigments are found in the contents of the intestine. The transformation of bilirubin into urobilin does not occur, as previously stated, in the small, but in the large intestine in man.

As under normal conditions no putrefaction, or at least none worth mentioning, occurs in the small intestine, and as often nearly all the protein of the food is absorbed, it follows that ordinarily it is the secretions and cells rich in proteins which undergo putrefaction. That the secretions rich in proteins are destroyed in putrefaction in the intestine

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[1] Wien. Sitzungsber., 44.
[2] Zeitschr. f. Biologie., 20 and 24.
[3] Zeitschr. f. physiol. Chem., 12.

follows from the fact that putrefaction may also continue during complete fasting. From the observations of MÜLLER[1] upon CETTI it was found that the elimination of indican during starvation rapidly decreased and after the third day of starvation it had entirely disappeared, while the phenol elimination, which at first decreased so that it was nearly minimum, increased again from the fifth day of starvation, and on the eighth or ninth day it was three to seven times as much as in man under ordinary circumstances. In dogs, on the contrary, the elimination of indican during starvation is considerable, but the phenol elimination is slight. Among the secretions which undergo putrefaction in the intestine, the pancreatic juice, which putrefies most readily, takes first place.

From the foregoing facts it must be concluded that the products formed by the putrefaction in the intestine are in part the same as those formed in digestion. The putrefaction may be of benefit to the organism in so far as the formation of such products as proteoses, peptones, polypeptides and amino-acids is concerned. The question has indeed been asked (PASTEUR), Is digestion possible without micro-organisms? NUTTAL and THIERFELDER have shown that guinea-pigs, removed from the uterus of the mother by Cæsarian section, could with sterile air digest well and assimilate sterile food (milk and crackers) in the complete absence of bacteria in the intestine, and developed normally and increased in weight. SCHOTTELIUS[2] has arrived at other results by experiments with hens. The chickens, hatched under sterile conditions, kept in sterile rooms and fed with sterile food, had continuous hunger and ate abundantly, but soon died, in about the same time as a starving chicken. On mixing with the food, at the proper time, a variety of bacteria from hen feces, they gained weight again and recovered.

The bacterial action in the intestinal canal is, at least in certain cases, as with food rich in cellulose, necessary, and it acts in the interest of the organism. This action may, by the formation of further cleavage products, involve a loss of valuable material to the organism, and it is therefore important that putrefaction in the intestine be kept within certain limits. If an animal is killed while digestion in the intestine is going on, the contents of the small intestine give out a peculiar but not putrescent odor. Also the odor of the contents of the large intestine is far less offensive than a putrefying pancreas infusion or a putrefying mixture rich in protein. From this one may conclude that putrefaction in the intestine is ordinarily not nearly so intense as outside of the organism.

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[1] Berlin. klin. Wochenschr., 1887.

[2] Nuttal and Thierfelder, Zeitschr. f. physiol. Chem., **21** and **22**; Schottelius, Arch. f. Hygiene, **34**, **42**, and **67**.

It seems thus to be provided, under physiological conditions, that putrefaction shall not proceed too far, and the factors which here come into consideration are probably of different kinds. Absorption is undoubtedly one of the most important of them, and it has been proved by actual observation that the putrefaction increases, as a rule, as the absorption is checked and fluid masses accumulate in the intestine. The character of the food also has an unmistakable influence, and it seems as if a large quantity of carbohydrates in the food acts against putrefaction (HIRSCHLER[1]). It has been shown by PÖHL, BIERNACKI, ROVIGHI, WINTERNITZ, SCHMITZ, and others[2] that milk and kephir have a specially strong preventive action on putrefaction. This action is not due to the casein, but chiefly to the lactose and also in part to the lactic acid.

A specially strong preventive action on putrefaction has been ascribed for a long time to the bile. This anti-putrid action does not exist in neutral or faintly alkaline bile, which itself easily putrefies, but to the free bile-acids, especially taurocholic acid (MALY and EMICH, LINDBERGER[3]). There is no question that the free bile-acids have a strong preventive action on putrefaction outside of the organism, and it is therefore difficult to deny such an action in the acid reacting contents of the intestine. Notwithstanding this, the anti-putrid action of the bile in the intestine is not considered by certain investigators (VOIT, RÖHMANN, HIRSCHLER and TERRAY, LANDAUER and ROSENBERG[4]) as of great importance.

Biliary fistulas have been established so as to study the importance of the bile in digestion (SCHWANN, BLONDLOT, BIDDER and SCHMIDT,[5] and others). As a result it has been observed that with fatty foods an imperfect absorption of fat regularly takes place and the excrement contains, therefore, an excess of fat and has a light-gray or pale color. The extent of deviation from the normal after the operation is essentially dependent upon the character of the food. If an animal is fed on meat and fat, then the quantity of food must be considerably increased after the operation, otherwise the animal will become very thin, and

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[1] Hirschler, Zeitschr. f. physiol. Chem., **10**; Zimnitzki, *ibid.*, **39** (literature).

[2] Schmitz, *ibid.*, **17**, 401, which gives references to the older literature, and **19**. See also Salkowski, Centralbl. f. d. med. Wiss., 1893, 467, and Seelig, Virchow's Arch., **126** (literature).

[3] Maly and Emich, Monatshefte, f. Chem., **4**; Lindberger, footnote 4, p. 506.

[4] Voit, Beitr. zur Biologie, Jubiläumschrift, Stuttgart, 1882; Röhmann, Pflüger's Arch. **29**; Hirschler and Terray, Maly's Jahresber., **26**; Landauer, Math, u. Naturw. Ber. aus Ungarn, **15**; Rosenberg, Arch. f. (Anat. u.) Physiol., 1901.

[5] Schwann, Müller's Arch. f. Anat. u. Physiol., 1844; Blondlot, cited from Bidder and Schmidt, Verdauungssäfte, etc., 98.

indeed die with symptoms of starvation. In these cases the excrement has the odor of carrion, and this was considered a proof of the action of the bile in checking putrefaction. The emaciation and the increased want of food depend, naturally, upon the imperfect absorption of the fats, whose high calorific value is reduced and must be replaced by the taking up of larger quantities of other nutritive bodies. If the quantity of proteins and fats be increased, then the latter, which can be only incompletely absorbed, accumulate in the intestine. This accumulation of the fats in the intestine only renders the action of the digestive juices on proteins more difficult, and thus increases the amount of putrefaction. This explains the appearance of fetid feces, whose pale color is not due to a lack of bile-pigments, but to a surplus of fat (RÖHMANN, VOIT). If the animal is, on the contrary, fed on meat and carbohydrates, it may remain quite normal, and the leading off of the bile does not cause any increased putrefaction. The carbohydrates may be uninterruptedly absorbed in such large quantities that they replace the fat of the food, and this is the reason why the animal on such a diet does not become emaciated. As with this diet the putrefaction in the intestine is no greater than under normal conditions even though the bile is absent, it would seem that the bile in the intestine exercises no preventive action on putrefaction.

To this conclusion the objection may be made that the carbohydrates, which are capable of checking putrefaction, can, so to speak, undertake the anti-putrid action of the bile. But as there are also cases (in dogs with biliary fistula) where the intestinal putrefaction is not increased with exclusive meat diet,[1] it is maintained that the absence of bile in the intestine, even by exclusive carbohydrate food, does not always cause an increased putrefaction.

Although the question as to the manner in which the putrefactive processes in the intestine under physiological conditions are kept within certain limits cannot be answered positively, still it may be asserted that the faint acid reaction, and the absorption of water, and the relatively rapid movement, of the contents of the small intestine and their absorption, are important factors.

That the acid reaction in the intestine has a preventive influence on the putrefactive processes follows from the existing relation between the degree of acidity of the gastric juice and the putrefaction in the intestine. Since the investigations and observations of KAST, STADELMANN, WASBUTZKI, BIERNACKI and MESTER had proved that an increased putrefaction in the intestine occurred when the quantity of hydrochloric acid in the gastric juice was diminished or deficient,

[1] See Hirschler and Terray, l. c.

SCHMITZ [1] has shown in man that on the administration of hydrochloric acid, producing a hyperacidity of the gastric juice, the putrefaction in the intestine may be checked. The question arises whether the reaction in the small intestine is always acid and whether the acidity is strong enough to prevent putrefaction. In this connection it must be recalled that the acidity of the contents of the small intestine is not due to hydrochloric acid, but chiefly to organic acids, acid salts, and free carbon dioxide. There are several observations as to the reaction of the intestinal contents, by MOORE and ROCKWOOD, MOORE and BERGIN, MATTHES and MARQUARDSEN, I. MUNK, NENCKI and ZALESKI, HEMMETER,[2] although they are somewhat contradictory. From these reports one can conclude that the reaction may vary not only among different animals, but also in the same animals under varying conditions. There is no doubt that the acid reaction in many cases is due to the presence of organic acids. On testing with various indicators it has been shown that sometimes the upper parts, and often the lower parts, are acid, due to acid salts such as $NaHCO_3$ and free CO_2, and finally that in certain animals the intestinal contents are alkaline throughout. The question how, under these conditions, putrefaction is excluded, and how the acidity of the gastric contents influences the intestinal putrefaction, cannot be explained. It is very probable that the bacterial flora of the intestine is of very great importance and it is possible, as BIENSTOCK admits, that the explanation lies in an antagonistic bacterial action and that the carbohydrates, especially lactose, which retard putrefaction, form a good nutritive media for those bacteria which destroy the putrefactive producers or retard their development. According to HOROWITZ an unequal division of the various bacteria occurs in dogs in the different parts of the intestine and certain varieties of bacteria occur in greater quantities than others, according to the kind of food taken. The influence of the kind of food upon the intestinal flora has also been studied by KENDALL. Perhaps, also, agreeing with the experience of CONRADI and KURPJUWEIT,[3] the toxins produced by the intestinal bacteria may, by their antiseptic action, keep the putrefactive processes in the intestine within bounds.

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[1] Zeitschr. f. physiol. Chem., **19**, 401, which includes all the pertinent literature.

[2] Moore and Rockwood, Journ. of Physiol., **21**; Moore and Bergin, Amer. Journ. of Physiol., **3**; Matthes and Marquardsen, Maly's Jahresber., **28**; Munk, Centralbl. f. Physiol., **16**; Nencki and Zaleski, Zeitschr. f. physiol. Chem., **27**; Hemmeter, Pflüger's Arch., **81**.

[3] Bienstock, Arch. f. Hygiene, **29**; Horowitz. Zeitschr. f. physiol. Chem., **52**; Kendall, Journ. of biol. Chem. **6**, 499 (1909); Conradi and Kurpjuweit, Münch. med. Wochenschr., 1905.

Feces. It is evident that the residue which remains after complete digestion and absorption in the intestine must be different, both qualitatively and quantitatively, according to the variety and quantity of the food. In man the quantity of excrement from a mixed diet is 120–150 grams, with 30–37 grams of solids, per twenty-four hours, while the quantity from a vegetable diet, according to VOIT,[1] was 333 grams, with 75 grams of solids. With a strictly meat diet the excrement is scanty, pitch-like, and black. The scanty feces in starvation have a similar appearance. A large quantity of coarse bread yields a great amount of light-colored excrement. In these cases the feces are also habitually poorer in nitrogen than after food rich in protein. The individuality also plays an important rôle in the utility of the food and the formation of feces (SCHIERBECK[2]). If there is a large proportion of fat, it takes a lighter clay-like appearance. The decomposition products of the bile-pigments seem to play only a small part in the normal color of the feces.

The constituents of the feces are of different kinds. In the excrement are found digestible or absorbable constituents of the food, such as muscle fibers, connective tissues, lumps of casein, grains of starch, and fat, which have not had sufficient time to be completely digested or absorbed in the intestinal tract. In addition the excrement contains indigestible bodies, such as the remains of plants, keratin substances, and others; also form-elements originating from the mucous coat and the glands; constituents of the different secretions, such as mucin, cholic acid, dyslysine, and cholesterin (koprosterin or stercorin), purine bases,[3] and enzymes; mineral bodies of the food and the secretions; and, lastly, products of putrefaction or of digestion, such as skatol, indol, volatile fatty acids, purine bases, lime, and magnesia soaps. Occasionally, also, parasites of different kinds occur; and lastly, the excrement contains micro-organisms of various species.

That the mucous membrane of the intestine by its secretion and by the abundant quantity of detached epithelium contributes essentially to the formation of feces follows from the discovery first made by L. HERMANN and substantiated by others,[4] that a clean, isolated loop

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[1] Zeitschr. f. Biologie, **25**, 264.

[2] Arch. f. Hygiene, **51**.

[3] In regard to the purine bases in feces, see Hall, Journ. of Path. and Bacteriol., **9**; Schittenhelm, Arch. f. klin. Med., **81**. Schittenhelm and Krüger, Zeitschr. f. physiol. Chem., **45**.

[4] Hermann, Pflüger's Arch., **46**. See also Ehrenthal, *ibid.*, 48; Berenstein, *ibid.*, **53**; Klecki, Centralbl. f. Physiol., **7**; 736, and F. Voit, Zeitschr. f. Biologie, **29**; v. Moraczewski, Zeitschr. f. physiol. Chem., **25**; F. Lippich, Prager med. Wochenschr., **82**.

we often find in the large intestine, balls similar to the so-called hair-balls (see below). Such calculi contain calcium and magnesium phosphate (about 70 per cent), oat-bran (15–18 per cent), soaps and fat (about 10 per cent). Concretions which contain very much fat (about 74 per cent) occasionally occur, and those consisting of fibrin clots, sinews, or pieces of meat incrusted with phosphates are also rare.

Intestinal calculi often occur in animals, especially in horses fed on bran. These calculi, which attain a very large size, are hard and heavy (as much as 8 kilos) and consist in great part of concentric layers of ammonium-magnesium phosphate. Another variety of concrements which occur in horses and cattle consists of gray-colored, often very large, but relatively light stones which contain plant residues and earthy phosphates. Stones of a third variety are sometimes cylindrical, sometimes spherical, smooth, shining, brownish on the surface, consisting of matted hairs and plant-fibers, and termed *hair-balls*. The so-called "ÆGAGROPILÆ," which occur in the ANTILOPE RUPICAPRA, belong to this group, and are generally considered as nothing else than the hair-balls of cattle.

The so-called *oriental bezoar-stone* also belongs to the intestinal concrements, and probably originates from the intestinal tract of the CAPRA ÆGAGRUS and ANTELOPE DORCAS. There may exist two varieties of bezoar-stones. One is olive-green, faintly shining and formed of concentric layers. On heating it melts with the development of an aromatic odor. It contains as chief constituent LITHOFELLIC ACID, $C_{20}H_{36}O_4$, which is related to cholic acid, and besides this a bile-acid, LITHOBILIC ACID. The others are nearly blackish brown or dark green, very glossy, consisting of concentric layers, and do not melt on heating. They contain as chief constituent ellagic acid, a derivative of gallic acid, of the formula $C_{14}H_6O_8$, which, according to GRAEBE,[1] is the dilactone of hexaoxydiphenyldicarboxylic acid, and which gives a deep-blue color with an alcoholic solution of ferric chloride. The last-mentioned bezoar-stone originates, to all appearances, from the food of the animal.

Ambergris is generally considered an intestinal concrement of the sperm whale. Its chief constituent is *ambrain*, which is a non-nitrogenous substance perhaps related to cholesterin. Ambrain is insoluble in water and is not changed by boiling alkalies. It dissolves in alcohol, ether, and oils.

VI. ABSORPTION.

The contents of the intestine are gradually pushed onward by the peristalsis or rhythmical movement of the intestinal musculature, but the mechanism is not well known.[2] By these processes the intestinal contents are intimately mixed and the constituents of the food which are valuable to the organism are transformed, in the manner previously mentioned, so that they are adaptable for the processes of absorption. In discussing the absorption processes we must treat of the form into which the different foods are changed before absorption, of the manner in which this is accomplished, and lastly, of the forces which act in these processes.

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[1] Ber. d. d. chem. Gesellsch., **36**.

[2] See Cannon, Amer. Journ. of Physiol., **6**, **12**, **29**; Magnus, Pflüger's Arch., **102**, **103**, **108**, **111**; Baumstark and Cohnheim, Zeitschr. f. physiol. Chem., **65**.

Before we can answer the question as to the form in which the proteins are absorbed from the intestinal canal, it is of interest to learn whether the animal body can, perhaps, also utilize such proteins as are introduced intravenously, subcutaneously, or into a body-cavity, i.e., evading the intestinal canal, or, as it is called *parenteral*.

Since the first investigations of ZUNTZ and v. MERING on this subject, several experimenters[1] have shown, without any doubt, that the animal body can more or less completely utilize different, parenterally introduced proteins, although different varieties of animals show a difference in this regard. Still we do not know where and how these foreign proteins are changed and assimilated, but CRAMER ascribes great importance to the leucocytes in this regard. See ABDERHALDEN'S experiment given on page 54.

That the animal body can also assimilate not previously digested or split proteins introduced directly into the intestine has been shown by BRÜCKE, BAUER and VOIT, EICHHORST, CZERNY and LATSCHENBERGER, VOIT and FRIEDLÄNDER, and others.[2] In the experiments of the two last-mentioned investigators neither casein (as milk) nor hydrochloric-acid myosin or acid albuminate (in acid solution) was absorbed, while, on the contrary, about 21 per cent of ovalbumin or seralbumin and 69 per cent of alkali albuminate (dissolved in alkali) were absorbed. MENDEL and ROCKWOOD, on the contrary, in experiments with casein and edestin in the living intestinal loop, could prove only the slightest absorption on excluding digestion as completely as possible, while the corresponding proteoses were abundantly absorbed.

It is difficult to decide in these experiments as to how far the proteins were taken up in an actually unchanged or partly modified form. The alimentary albuminaria, observed repeatedly after the introduction of large quantities of protein into the intestinal canal, indicates an absorption of undigested protein under certain circumstances. To decide this question the biological method, using the precipitine reaction, has been made use of, and ASCOLI and VIGNO,[3] using this method, claim to

[1] Zuntz and v. Mering, Pflüger's Arch., **32**; Neumeister, Verh. d. phys.-med. Gesellsch. zu Würzburg, 1889, and Zeitschr., f. Biologie, **27**; Friedenthal and Lewandowsky, Arch. f. (Anat. u.) Physiol., 1899; Munk and Lewandowsky, *ibid.*, 1899, Supp.; Oppenheimer, Hofmeister's Beiträge, **4**; Mendel and Rockwood, Amer. Journ. of Physiol., **12**; Heilner, Zeitschr. f. Biol., **50**, and Münch, med. Wochenschr., **49**; Cramer, Journ. of Physiol., **37**, with Pringle, *ibid.*; Rona and Michaelis, Pflüger's Arch., **128** and **124**; v. Körösy, Zeitschr. f. physiol. Chem. **62**, 68 (1909), **69**, 313 (1910).

[2] Brücke, Wien. Sitzungsber., **59**; Bauer and Voit, Zeitschr. f. Biologie, **5**; Eichhorst, Pflüger's Arch., **4**; Czerny and Latschenberger, Virchow's Arch., **59**; Voit and Friedländer, Zeitschr. f. Biologie, **33**. Contradictory observations can be found in Keller, Beitr. z. Frage d. Resorption im Dickdarm. Inaug.-Dissert. Breslau, 1909.

[3] Zeitschr. f. Physiol. Chem., **39**.

have shown the passage of non-modified protein into the blood and lymph (page 66). Based upon many investigations on this subject we can consider it possible that under certain circumstances, as on flooding the intestinal canal with protein, with a greater permeability of the intestinal wall, as in new-born and sucking animals, and with a diminished modification by the gastric juice, a passage of non-modified protein may take place in the blood-vessels, but that under normal conditions this is not the case, or at least does not take place to any mentionable degree. As a rule, the absorption of protein follows a modification of it. In this connection the experiments of ORNI [1] are of interest which show that the dog's intestine takes up the serum of the dog but not that of the ox or horse. In regard to the previously split proteins the question arises whether the proteins are chiefly absorbed as proteoses or peptones or as simpler atomic complexes.

According to the earlier investigations of LUDWIG and SCHMIDT-MÜLHEIM, as well as those of MUNK and ROSENSTEIN,[2] it is generally agreed that the products of protein digestion do not pass into the blood through the lymph vessels, but through the intestinal capillaries. The question of the absorption of these products resolves itself into the form in which they are taken up by the intestine and the form in which they pass into the blood.

It was mentioned above that proteoses and peptones as well as non-biuret-giving products and amino-acids have been found in the contents of the intestine. The amino-acids occur to a less extent than the proteoses and peptones. This may indicate that the amino-acids are more abundantly formed, but also more quickly absorbed, but it may also indicate that the amino-acids are produced to a slight extent only, in the intestinal contents. There is no doubt that the amino-acids can be absorbed as such, but there is still another question, namely, whether the proteoses and peptones are absorbed as such or only after a previous cleavage into amino-acids.

NOLF and HONORÉ found, what was later substantiated by ZUNZ,[3] that the proteoses and peptones disappear more quickly from the intestine than the non-biuret-giving products. This does not prove that the proteoses are absorbed as such, but rather against such a view. A more direct proof for the absorption of the non-split proteoses lies in the fact, as shown by NOLF, that the proteoses when introduced in

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[1] Pflüger's Arch. **126**, 428 (1909).

[2] Schmidt-Mülheim, Arch. f. (Anat. u.) Physiol., 1877; Munk and Rosenstein. Virchow's Arch., **123**.

[3] Nolf and Honoré, Arch. internat. de Physiol., 1905; Nolf, Journ. de Physiol. et Pathol. gén., 1907; Zunz, Mémoires cour., etc., Acad. Roy. Méd., Belg., **20**, Fasc. 1.

large quantities in the intestine pass in small amounts into the blood. Another proof is the findings of BORCHARDT,[1] that after feeding dogs with not too large amounts of elastin, the passage of a proteose, the hemielastose, could be detected in the blood. Attention must also be called to the fact that according to HOFMEISTER[2] the walls of the stomach and intestine are the only parts of the body in which proteoses and peptones occur during digestion.

We have reason for believing that the proteoses, as well as their cleavage products, are taken up by the intestine, and if this is the case the next question to be answered is, in what form do these bodies leave the intestine and pass into the blood?

In order to decide this question the blood has been repeatedly tested in regard to the quantity of proteoses. As seen on page 264 this has led to very contradictory results, and if we exclude those exceptional cases where a large quantity of proteose was introduced into the intestine at once, then we can say that the occurrence of proteoses in the blood, or at least in the blood-plasma, has not been positively shown under physiological conditions.[3] It can also be said that such investigations do not prove much because of the large quantity of blood passing through the intestine for a given time, and the quantity of proteose must be so small, so that when divided in the entire blood it can hardly be detected. It is therefore of interest that neither amino-acids nor proteoses were found in the blood after cutting out several organs or groups of organs so that the blood circulated only through the intestinal canal, heart, lungs, pancreas and intercostal muscles (KUTSCHER and SEEMANN, v. KÖRÖSY[4]).

We are therefore obliged to consider that the proteoses and amino-acids are transformed in the intestinal walls in some manner or other. Such a belief, especially applied to the proteoses, coincides with the observations of HOFMEISTER, that the proteoses occurring in the mucous membrane during digestion disappear at the temperature of the room from the removed, but still apparently living, mucous membrane after a certain time. This also coincides well with the observations of LUDWIG and SALVIOLI.[5] These investigators introduced a peptone solution into a double-ligatured, isolated piece of the small intestine, which was kept alive by passing defibrinated blood through it, and observed that the

[1] In regard to the literature on proteoses in the blood see Chapter V, footnotes 1, 2 and 3, p. 264.

[2] Zeitschr f. physiol. Chem., **6**, and Arch. f. exp. Path. u. Pharm., **19**, **20**, **22**.

[3] See footnote 1.

[4] Kutscher and Seemann, Zeitschr. f. physiol. Chem., **24**; v. Körösy, *ibid.*, **57**.

[5] Arch. f. (Anat. u.) Physiol., 1880, Supplbd. See also Cathcart and Leathes, Journ. of Physiol., **33**.

peptone disappeared from the intestine, but that the blood passing through did not contain any peptone.

What becomes of the amino-acids in the intestinal wall? KUTSCHER and SEEMANN have shown that the crystalline cleavage products are so transformed in the intestinal wall that they cannot be detected. We have here to think of two possibilities: The amino-acids are either further split or they are used in synthesis (of proteins?).

It is a long-known fact that with the digestion and absorption an increased elimination of nitrogen in the urine goes hand in hand. The quantity of nitrogen eliminated in the urine after partaking of protein corresponded, according to ASHER and HAAS,[1] to 65 per cent of the nitrogen introduced. It is hardly credible that this elimination of nitrogen depends upon an increased destruction of body protein, and it is more probable that this represents decomposed food-protein. But according to NENCKI and ZALESKI[2] an abundant formation of ammonia occurs in the cells of the digestive apparatus after a rich protein diet, so we must consider the possibility that a considerable part, perhaps the very greatest part, of the amino-acids are deamidized in the intestinal wall. The other part of the amino-acids may be used in the syntheses to be mentioned below. Such a partial deamidization of the digestive products has been shown by COHNHEIM[3] in his absorption experiments with the fish intestine.

The proteoses taken up by the intestinal mucosa, if this does take place, can naturally undergo a further conversion into amino-acids in the walls of the intestine. Still there are other possibilities. A direct utilization of the proteoses in the synthesis of the proteins in the intestine is not very probable, but on the contrary it is more probable that the proteoses, in order to undergo further cleavage or further utilization, are taken up by the leucocytes and carried off. HOFMEISTER has advocated such a possibility for a long time. HEIDENHAIN raised objections to this suggestion in which he called attention to the disproportion between the number of leucocytes and the large quantity of peptones (proteoses) to be absorbed, but at that time the deep cleavage of a great part of the protein into amino-acids was not known. Recently PRINGLE and CRAMER[4] urged the theory of the importance of the leucocytes, and the observations of INAGAKI[5] also show the possibility

[1] Bioch. Zeitschr., **12**.

[2] Arch. des scienc. biol. de St. Pétersbourg, **4**; Arch. f. exp. Path. u. Pharm., **37**; see also Salaskin, Zeitschr. f. Physiol. Chem., **25**.

[3] Zeitschr. f. physiol. Chem., **59**.

[4] Hofmeister, l. c.; Heidenhain, Pflüger's Arch., **43**; Pringle and Cramer, Journ. of Physiol., **37**.

[5] Zeitschr. f. physiol. Chem., **50**.

of the leucocytes taking up the proteoses and fixing them, it seems, in the cell substance.

It is for the present impossible to say with certainty whether or not and to what extent the proteoses, as such, are absorbed and to give their further fate thereafter in the intestine. The present view is probably as follows: That they do not pass as such into the blood, and that they are transformed into amino-acids in part in the intestinal contents and in part in the intestinal mucosa, and then from these amino-acids the coagulable proteins are constructed by synthesis. In support of the theory of a protein synthesis from amino-acids we have a series of experiments where deeply split or completely split proteins were fed. In these experiments by LOEWI, HENDERSON and DEAN, HENRIQUES and HANSEN, and especially by ABDERHALDEN and his co-workers [1] on dogs, mice and rats, it was possible to keep the animals in nitrogenous equilibrium or indeed nitrogen retention for a long time with the cleavage products of proteins besides non-nitrogenous foodstuffs and salts. According to the recent experiments of ABDERHALDEN the organism can build up proteins from amino-acids when the individual amino-acids are supplied in proportions as they exist in the cell proteins. Certain, sometimes absent amino-acids seem to be capable of being produced within the organism (for example, glycocoll, proline) while other (tryptophane) cannot be produced. This explains why gelatine which does not contain any tryptophane cannot replace protein in the food.

The results of the experiments are generally considered as proof of the ability of the animal body to construct proteins from amino-acids by synthesis, and in the present state of our knowledge we can hardly draw other conclusions from them or advance any simpler theory.

Where does the protein synthesis take place? If it were positively sure that the amino-acids did not pass into the blood then we would have transferred this synthesis to the intestinal walls. Otherwise we must think in the first place of the liver; but this organ does not seem to play an important rôle in this synthesis. ABDERHALDEN and LONDON [2] made an experiment on a dog with an ECK fistula (see page 397), feeding the dog with decomposed protein, and they found that this animal behaved exactly like a normal animal, as it was kept for eight days not only in nitrogenous equilibrium but also in nitrogen retention. On

[1] Loewi, Arch. f. exp. Path. u. Pharm., **48**. See also Henderson and Dean, Amer. Journ. of Physiol., **9**; Abderhalden and Rona, Zeitschr. f. physiol. Chem., **42**, **44**, **47**, and **52**; Henriques and Hansen, *ibid.*, **43**, **49**; Henriques, *ibid.*, **54**; Abderhalden with Olinger, *ibid.*, **57**, with Messner and Windrath, *ibid.*, **59**; Abderhalden, *ibid.*, **77**, 22, 78, 1 (1912).

[2] Zeitschr. f. physiol. Chem., **54**.

the other hand it is not possible to deny the importance of the liver for the protein syntheses. As EMBDEN and his collaborators have shown on perfusing the liver containing a large amount of glycogen, that *d*-alanine was formed and EMBDEN explains this formation by a destruction of glucose or lactic acid and pyroracemic acid. With experiments with blood perfusion of the liver, α-amino-acids are formed from the ammonium salts of the corresponding α-keto-acids. The combination $NH_4.O.CO.CO.R$ passes into $HO.CO.CH(NH_2).R$. The cleavage products of the carbohydrates can be converted in the liver into characteristic constituents of the protein molecule.[1] In this connection we must here mention the experiments of LÜTHJE[2] in which he found a nitrogen retention after feeding only one amino-acid with abundance of carbohydrate.

What kind of protein is formed in the synthesis? This we do not know. ABDERHALDEN'S belief is that it is plasma protein, which, as is well known, is the same in each animal independent of the kind of protein introduced with the food and from which the cells of the body then create the further protein material. Objections can be raised against this hypothesis, but still it is worth consideration. In favor of this we can also add that according to the investigations of FREUND and v. KÖRÖSY[3] the blood coming from the intestine during digestion is richer in coagulable protein than other blood, and also that this protein, FREUND asserts, belongs to the globulin group. This globulin, according to FREUND and TOEPFER, is not identical with the ordinary serglobulin mixture, but is a pseudoglobulin formed in the intestine from the food protein by synthesis, and which is more easily decomposed or further utilized in the liver and other organs. Further research in this direction is necessary, as we have other investigations which are essentially different. If a re-formation of coagulable proteins takes place from amino-acids during digestion, it is to be expected that a relatively greater quantity of coagulable protein should occur in the mucosa of the digesting intestine as compared with the non-digesting intestine. PRINGLE and CRAMER, by a method which requires confirmation, claim that in the digesting animal (cat), the blood, and to a still higher degree the intestinal mucosa, and especially the lymph nodes of the intestine, are richer in non-coagulable protein than the starving animal, a condition which is related to the rôle of the leucocytes in the

[1] Bioch. Zeitschr. **29**, 423 (1910); **38**, 393, 407, 414 (1911); **45**, 1–207 (1912); summary, **45**, 201.

[2] Pflüger's Arch. **113**, 547 (1906).

[3] v. Körösy, Zeitschr. f. physiol. Chem., **57**; Freund, Zeitschr. f. exp. Path. u. Therap., **4**; G. Toepfer and Freund, and Toepfer, *ibid.*, **3**; Pringle and Cramer, Journ. of Physiol., **37**.

protein assimilation. This question of the absorption of proteins in the intestine is still unexplained in many directions.

The extent of the protein absorption is dependent essentially upon the kind of food introduced, since as a rule the protein substances from an animal source are much more completely absorbed than from a vegetable source. As proof of this the following observations are given: In his experiments on the utilization of certain foods in the intestinal canal of man, RUBNER found that with an exclusively animal diet, on partaking of an average of 738–884 grams of fried meat, or 948 grams of eggs per day, the nitrogen deficit with the excrement was only 2.5–2.8 per cent of the total nitrogen introduced. With a strictly milk diet the results were somewhat unfavorable, since after partaking of 4100 grams of milk the nitrogen deficit increased to 12 per cent. The conditions are quite different with vegetable food, as shown by the researches of MEYER, RUBNER, HULTGREN and LANDERGREN, who made experiments with various kinds of rye bread and found that the loss of nitrogen through the feces amounted to 22–48 per cent. Experiments with other vegetable foods, and also the investigations of SCHUSTER, CRAMER, MEINERT, MORI,[1] and others on the utilization of foods with mixed diets, have led to similar results. With the exception of rice, wheat bread, and certain very finely divided vegetable foods, it is found in general that the nitrogen deficit by the feces increases with a larger quantity of vegetable material in the food.

The reason for this is manifold. The large quantity of cellulose frequently present in vegetable foods impedes the absorption of proteins. The greater irritation produced by the vegetable food itself or by the organic acids formed in the fermentation in the intestinal canal causes a more violent peristalsis, which drives the contents of the intestine faster than otherwise along the intestinal canal. Another and most important reason is the fact that a part of the vegetable protein substances seems to be indigestible, and because of the difficultly digestible vegetable food, a large quantity of digestion fluids containing nitrogen is secreted.

In speaking of the functions of the stomach we stated that after the removal or excision of this organ, an abundant digestion and absorption of proteins may take place. It is therefore of interest to learn how the digestion and absorption of proteins go on after the extirpation of the second protein-digesting organ, the pancreas. In this connection

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[1] Rubner, Zeitschr. f. Biologie, **15**; Meyer, *ibid.*, **7**; Hultgren and Landergren, Nord. med. Arch., **21**; Schuster, in Voit's "Untersuch. d. Kost," etc., 142; Cramer, Zeitschr. f. physiol. Chem., **6**; Meinert, "Ueber Massenernährung," Berlin, 1885; Kellner and Mori, Zeistchr. f. Biologie, **25**.

there are the observations on animals after complete or partial extirpation of the gland by MINKOWSKI and ABELMANN, SANDMEYER, V. HARLEY, after destroying the gland by ROSENBERG, and also in man after closing the pancreatic duct by HARLEY and DEUCHER. In all these cases such discrepancy of figures has resulted for the utilization of the proteins—between 80 per cent after the apparently complete exclusion of pancreatic juice in man (DEUCHER) and 18 per cent after extirpation of the gland in dogs (HARLEY)—that one can hardly draw any clear conception as to the extent and importance of the trypsin digestion in the intestine. That on completely preventing the entrance of pancreatic juice only a slight diminution in the protein absorption takes place follows from the researches of LOMBROSO and NIEMANN.[1] In order to understand, in these cases, why the digestion and absorption took place so abundantly it would be of interest to know how other digestion fluids act substitutingly. In this regard ZUNZ and MAYER[2] found that in dogs (meat digestion) the tying of the pancreatic passages is essentially compensated for by an increased secretion of pepsin and other proteolytic enzymes, and that in this case the demolition of the protein in the stomach goes further than in a normal animal.

The carbohydrates are, it seems, chiefly absorbed as monosaccharides. Glucose, fructose, and galactose are probably absorbed as such. The two disaccharides, saccharose and maltose, ordinarily undergo an inversion in the intestinal tract and are converted into glucose and fructose. Lactose is also, at least in certain animals, inverted in the intestine. In other mature animals, on the contrary, if the lactase formation is not excited by milk food, the sugar is not inverted or only to a slight extent (VOIT and LUSK, WEINLAND, PORTIER, RÖHMANN and NAGANO), and it probably is absorbed as such in these animals if it does not undergo fermentation, or, as RÖHMANN and NAGANO[3] assumed, if it is not transformed in the intestinal mucosa in some unknown way. An absorption of non-inverted carbohydrates is not improbable, and according to OTTO and v. MERING the portal blood contains, after a carbohydrate diet, besides glucose, a dextrin-like carbohydrate. MOSCATI[4]

[1] Abelmann, "Ueber die Ausnützung der Nahrungsstoffe nach Pankreasexstirpation" (Inaug.-Dissert. Dorpat, 1890), cited from Maly's Jahresber., **20**; Sandmeyer, Zeitschr. f. Biologie, **31**; Rosenberg, Pflüger's Arch., **70**; Harley, Journ. of Pathol. and Bacteriol., 1895; Deucher, Correspond. Blatt. f. Schweiz. Aerzte, **28**; Lombroso, Arch. f. exp. Path. u. Pharm., **60**; Niemann, Zeitschr. f. exp. Path. u. Therap., **5**; See also Brugsch and Pletnew, Zeitschr. f. exp. Path. u. Therap. **6**, 326.

[2] Mem. de l'acad. roy. de médic. de Belg., 18.

[3] Voit and Lusk, Zeitschr. f. Biologie, **28**; Röhmann and Nagano, Pflüger's Arch., **95**, which contains the references to the literature.

[4] Otto, see Maly's Jahresber., **17**; v. Mering, Arch. f. (Anat., 4.) Physiol., 1877; Moscati, Zeitschr. f. physiol. Chem., **50**

believes that when homogeneous starch solutions are injected intravenously or subcutaneously, the starch is taken up by the organs, namely the spleen, liver and lungs, and is utilized as the starch can be changed into glycogen. A part of the carbohydrates is destroyed by fermentation in the intestine, with the formation of lactic and acetic acids and other bodies.

The different varieties of sugars are absorbed with varying degrees of rapidity, but as a general thing absorption occurs very quickly. This absorption takes place more quickly in the upper part of the intestine than in the lower part (RÖHMANN, LANNOIS and LÉPINE, RÖHMANN and NAGANO [1]). It is generally admitted that the simpler sugars are more quickly absorbed than the disaccharides, while the reports as to the absorption of the disaccharides differ somewhat (HÉDON, ALBERTONI, WAYMOUTH REID, RÖHMANN and NAGANO). There seems to be no doubt that lactose is absorbed more slowly than the two other disaccharides. According to the extensive experiments of RÖHMANN and NAGANO, saccharose is absorbed more quickly than maltose. NAGANO [2] contends that the pentoses are absorbed more slowly than hexoses.

On the introduction of starch even in very considerable quantities into the intestinal tract no glucose passes into the urine, a condition which probably depends in this case upon the absorption and assimilation and the slow saccharification taking place simultaneously. If, on the contrary, large quantities of sugar are introduced at one time, then an elimination of sugar by the urine takes place, and this elimination of sugar is called *alimentary glycosuria*. In these cases the assimilation of the sugar and the absorption do not take place together.

That quantity of sugar to which we must raise the ingested substance in order to produce an alimentary glycosuria gives, according to HOFMEISTER,[3] the *assimilation limit* for that same sugar. This limit is different for various kinds of sugar; and it also varies for the same sugar not only in different animals, but also in different members of the same species, as also in the same individual under varying circumstances. In general it can be said that in regard to the ordinary varieties of sugar, such as glucose, fructose, galactose, saccharose, maltose, and lactose, the assimilation limit is highest for glucose and lowest for lactose. It must be admitted that with an overabundant quantity of sugars in the intestinal tract the disaccharides do not have sufficient time for their complete inversion, and this has been directly shown by

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[1] Lannois and Lépine, Arch. de physiol. (3), 1; Röhmann, Pflüger's Arch., 41; see also footnote 3, p. 532.

[2] In regard to the literature on the absorption of sugars, see footnote 3, p. 532.

[3] Arch. f. exp. Path. u. Pharm., 25 and 26.

RÖHMANN and NAGANO. It is, therefore, not remarkable that disaccharides, as well, have been found in the urine in cases of alimentary glycosuria.[1]

The investigations of LUDWIG and v. MERING and others have explained how the sugars enter into the blood-stream, namely, that they as well as other bodies soluble in water do not ordinarily pass over into the chylous vessels in measurable quantities, but are chiefly taken up by the blood in the capillaries of the villi, and in this way pass into the mass of the blood. These investigations have been confirmed by observations of I. MUNK and ROSENSTEIN [2] on human beings.

The reason why the sugars and other soluble bodies do not pass over into the chylous vessels in appreciable quantity is, according to HEIDENHAIN,[3] to be found in the anatomical conditions, in the arrangement of the capillaries close under the layer of epithelium. Ordinarily these capillaries find the necessary time for the removal of the water and the solids dissolved in it. But when a large quantity of liquid, such as a sugar solution, is introduced into the intestine at once, this is not possible, and in these cases a part of the dissolved bodies passes into the chylous vessels and the thoracic duct (GINSBERG and RÖHMANN [4]).

The passage of sugar into the urine, when at one time large quantities of sugar are taken and the assimilation limit is exceeded, can be best explained by the assumption that a part of the sugar escaped the liver and passed into the large circulation, or that the liver did not have time to retain the sugar and transform it into glycogen. According to the observations of de FILIPPI[5] upon dogs with ECK fistula, it seems as if the rôle of the liver in these cases is too highly estimated. An animal with ECK fistula could take an unlimited quantity of starch without glycosuria occurring. The assimilation limit was in these cases somewhat lower, but qualitatively they behave like normal animals and with increasing sugar supply they could also retain increasing quantities of sugar.

The introduction of larger quantities of sugar into the intestine at one time can readily cause a disturbance with diarrheal evacuations of the intestine. If the carbohydrate is introduced in the form of starch,

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[1] For the literature in regard to the passage of various kinds of sugars into the urine, see C. Voit. Ueber die Glykogenbildung, Zeitschr. f. Biologie, **28**, and F. Voit, footnote 1, p. 396. See also Blumenthal, Zur Lehre von der Assimilationsgrenze der Zuckerarten, Inaug.-Dissert. 1903, Strassburg and Brasch, Zeitschr. f. Biol., **50**.

[2] v. Mering. Arch. f. (Anat. u). Physiol., 1877; Munk and Rosenstein, Virchow's Arch. **123**.

[3] Pflüger's Arch., **43**, Suppl.

[4] Ginsberg, Pflüger's Arch., **44**; Röhmann, *ibid.*, **41**.

[5] Zeitschr. f. Biol., **49** and **50**.

then very large quantities may be absorbed without causing any disturbance, and the absorption may be very complete. RUBNER found the following: On partaking 508–670 grams of carbohydrates, as wheat bread, per day, the part not absorbed amounted to only 0.8–2.6 per cent. For peas, where 357–588 grams were eaten, the loss was 3.6–7 per cent, and for potatoes (718 grams) 7.6 per cent. CONSTANTINIDI found on partaking 367–380 grams of carbohydrates, chiefly as potatoes, a loss of only 0.4–0.7 per cent. In the experiments of RUBNER, as also of HULTGREN and LANDERGREN,[1] with rye bread the utilization of carbohydrates was less complete, and the loss in a few cases rose even to 10.4–10.9 per cent. It at least follows from the experiments made thus far that man can absorb more than 500 grams of carbohydrates per diem without difficulty.

We generally consider the pancreas as the most important organ in the digestion and absorption of amylaceous bodies, and it is a question how these bodies are absorbed after the extirpation of the pancreas. As on the absorption of proteins, so also on the absorption of starch, the observations have given variable results. In certain cases the absorption was not impaired, while in others it was, on the contrary, rather diminished, and with dogs devoid of pancreas it has been found that the absorption was decreased to 50 per cent of the starch partaken (ROSENBERG, CAVAZZANI[2]).

Emulsification used to be considered as of the greatest importance in the absorption of fats, and this emulsion occurs in the chyle on the introduction into the intestine of not only neutral fats, but also of fatty acids. The fatty acids do not exist as such in the emulsified fat of the chyle. The investigations of I. MUNK, later confirmed by others, have shown that the fatty acids undergo in great part a synthesis into neutral fats in the walls of the intestine, and are carried as such by the stream of chyle into the blood. This synthesis seems to take place in the mucous membrane (MOORE and others[3]).

The assumption that the fat is absorbed chiefly as an emulsion is partly based on the abundance of emulsified fat in the chyle after feeding with fat, and partly on the fact that a fat emulsion is often found in the intestine after such food. As an abundant cleavage of neutral

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[1] Rubner, Zeitschr. f. Biologie, **15** and **19**; Constantinidi, *ibid.*, **23**; Hultgren and Landergren, Nord. med. Arch. **21**.

[2] Cavazzani, Centralbl. f. Physiol., **7**. See footnote 1, p. 532; also Lombroso, Hofmeister's Beiträge, 8.

[3] Munk, Virchow's Arch., **80**. See also v. Walther, Arch. f. (Anat. u.) Physiol., 1890; Minkowski, Arch. f. exp. Path. u. Pharm., **21**; Frank, Zeitschr. f. Biologie, **36**; Moore, see Biochem. Centralbl., **1**, 741; Frank and Ritter, Zeitschr. f. Biologie, **47**; Noll, Pflüger's Arch. **136**.

fats occurs in the intestinal canal, and also as the fatty acids do not occur in the chyle as such, but as emulsified fat after a synthesis with glycerin into neutral fats, it is to be doubted whether the emulsified fat of the chyle originates from an absorption of emulsified fat in the intestine or from a subsequent emulsification of neutral fats formed synthetically. This doubt has greater warrant in the observation of FRANK [1] that the fatty-acid ethyl ester is extensively taken up from the intestine, not as such, but as split-off fatty acids from which then the neutral emulsified fats of the chyle are formed.

The assumption of an absorption of fats as an emulsion is inconsistent with the fact that an emulsion produced by means of soaps is not permanent in an acid liquid; hence we cannot consider as possible the presence of an emulsion in the intestine so long as it is acid. This difficulty is not too serious, as the reaction is often only due to carbonic acid and bicarbonates, and besides as found by KÜHNE and recently shown by MOORE and KRUMBHOLZ,[2] the proteins have a preserving action upon fat emulsions.

The earlier opinions as to fat absorption were, that fat was absorbed as soaps, soluble in water, as well as finely emulsified fat, and this last form was considered as of the greatest importance. This view has recently undergone essential modifications, due to the work of MOORE and ROCKWOOD, and especially to the extensive work of PFLÜGER.[3]

MOORE and ROCKWOOD have shown the great solvent action of the bile for fatty acids, and on continuing these investigations further, MOORE and PARKER have found that the bile increases the solubility of soaps in water, and can prevent their gelatinization, a fact which is of greater importance for the absorption of fats than the solubility of the fatty acids in bile. The quantity of lecithin in the bile is of great importance for the solubility therein of the fatty acids as well as the soaps. According to the above-mentioned investigators, the absorption of fat from the intestine is essentially dependent upon the solubility of the soaps and free fatty acids in the bile. The neutral fats are split and the free fatty acids are in part absorbed, dissolved as such by the bile, and in part combined with alkalies, forming soaps. Neutral fats are regenerated from the fatty acids, and the alkali set free from the soaps is secreted again into the intestine and used for the re-formation

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[1] Zeitschr. f. Biologie, 36.

[2] Kühne, Lehr. der physiol. Chem., 122; Moore and Krumbhols, Journ. of Physiol., 22.

[3] In regard to the recent literature on fat absorption, see the works of Pflüger, Pflüger's Arch., 80, 81, 82, 85, 88, 89, and 90, where the work of other investigators is cited and discussed. See also Croner, Bioch. Zeitschr. 23; Lombroso, Arch. di Fisiol. 5.

of soaps. According to CRONER the absorption of soaps occurs only in the lower parts of the small intestine.

The importance of the bile, the soaps, and the alkali carbonates has been closely studied, principally in the very thorough investigations of PFLÜGER. He has quantitatively determined the solvent power of the above-mentioned bodies—each alone as well as different mixtures of these—for the various fatty acids, and has closely studied the mode of action of the bile. From his investigations he has arrived at the conclusion that no unsplit fat is absorbed, that all fats, before their absorption, must first be split into glycerin and fatty acids, and that the bile, on account of its solvent power for soaps and fatty acids, is sufficient for the absorption of large quantities of fat eaten. The object of the formation of an emulsion is, according to this view, that the fat in this condition forms such a large surface for the action of the steapsin or the fat-splitting agents. The possibility that all the fat must be first split and that no unsplit fat is absorbed is, according to these researches, not to be denied.

The next question is whether all the fat or the greater part of it passes into the blood through the lymphatics and the thoracic duct. According to the researches of WALTHER and FRANK[1] on dogs, it seems that only a small part of the fats, or at least of the fatty acids fed, passes into the chylous vessels; but these observations can hardly be applied to the absorption of neutral fats, or to the absorption in man under normal circumstances. MUNK and ROSENSTEIN,[2] in their investigations on a girl with a lymph fistula, found 60 per cent of the fat ingested in the chyle, and of the total quantity of fat in the chyle only 4–5 per cent existed as soaps. On feeding with a foreign fatty acid, such as erucic acid, they found 37 per cent of the introduced body as neutral fat in the chyle. Not all the fat introduced is found in the chyle, and there is always a not inconsiderable part of the absorbed fat whose fate we are not able to follow.

The completeness with which fats are absorbed depends, under normal conditions, essentially upon the kind of fat. In this regard it is known, especially from the investigations of MUNK and ARNSCHINK,[3] that the varieties of fat with high melting-points, such as mutton-tallow, and especially stearin, are not so completely absorbed as the fats with low melting-points, such as hog- and goose-fat, olive-oil, etc. The kind of fat also has an influence on the rapidity of absorption, as MUNK and ROSENSTEIN found that solid mutton-fat was absorbed more slowly

[1] Walther, Arch. f. (Anat. u.) Physiol., 1890; Frank, *ibid.*, 1892.
[2] Virchow's Arch., **123**.
[3] Munk, Virchow's Arch., **80** and **95**; Arnschink, Zeitschr. f. Biologie. **26**.

than fluid lipanin. The extent of absorption in the intestinal tract is, under physiological conditions, very considerable. In the case of a dog investigated by VOIT it was found that out of 350 grams of fat (butter) partaken, 346 grams were absorbed from the intestinal canal, and according to the investigations of RUBNER[1] the human intestine can absorb over 300 grams of fat per diem. The fats are, according to RUBNER, much more completely absorbed when free, in the form of butter or lard, than when inclosed in cell-membranes, as in bacon.

CLAUDE BERNARD showed long ago with experiments on rabbits in which the ductus choledochus was made to open into the small intestine above the pancreatic duct, that after food rich in fats the chylous vessels of the intestine above the pancreas passages were transparent, while below they were milk-white, and also that the bile alone cannot produce an absorption of the emulsified fat without the pancreatic juice. DASTRE[2] has performed the reverse experiment on dogs. He tied the ductus choledochus and adjusted a biliary fistula so that the bile flowed into the intestine below the mouth of the pancreatic passages. On killing the animal after a meal rich in fat the chylous vessels were first found milk-white below the discharge of the biliary fistula. From this DASTRE draws the conclusion that a combined action of the bile and pancreatic juice is important in the absorption of fats—a conclusion which stands in accord with the experience of many others.

Through numerous observations of many investigators, such as BIDDER and SCHMIDT, VOIT, RÖHMANN, FR. MÜLLER, I. MUNK,[3] and others, it has been shown that the exclusion of the bile from the intestinal tract diminishes the absorption of fat to such an extent that only one-seventh to about one-half of the quantity of fat ordinarily absorbed undergoes absorption. In icterus with entire exclusion of the bile, a considerable decrease in the absorption of fat is noticed. As under normal conditions, so also in the absence of bile in the intestine, the lower melting parts of the fat are more completely absorbed than those which have a high melting-point. I. MUNK found in his experiments on dogs with lard and mutton-tallow that the absorption of the high-melting tallow was reduced twice as much as the lard on the exclusion of the bile from the intestine.

We also learn from the investigations of RÖHMANN and I. MUNK that in the absence of bile the relation between fatty acids and neutral fats is changed, namely, about 80–90 per cent of the fat existing in the

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[1] Voit, Zeitschr. f. Biologie, 9; Rubner, *ibid.*, 15.

[2] Arch. de Physiol. (5), 2.

[3] F. Müller, Sitzungsber. der phys.-med. Gesellsch. zu Würzburg, 1885; I. Munk, Virchow's Arch., 122. See also footnotes 4 and 5, p. 518.

feces consists of fatty acid, while under normal conditions the feces contain 1 part neutral fat to about 2–2½ parts free fatty acids. It is not possible to state how this increased quantity of fatty acids in the fat of the feces is produced upon the exclusion of the bile from the intestine.

There is no doubt that the bile is of great importance in the absorption of fats. Still there is also no doubt that rather considerable quantities of fat may be absorbed from the intestine in the absence of bile. What relation does the pancreatic juice bear to this fact?

Upon this point a rather large number of observations on animals have been made by ABELMANN and MINKOWSKI, SANDMEYER, HARLEY, ROSENBERG, HÉDON and VILLE, and also on man by FR. MÜLLER and DEUCHER.[1] In all of these investigations a more or less diminished absorption of fat was observed after the extirpation or destruction of the gland, or the exclusion of the juice from the intestine. The results are very diverse as to the extent of this diminution, as in certain cases no absorption of fat was observed, while, in other cases, a considerable absorption was noted in the same class of animal (dog) and even in the same animal. According to MINKOWSKI and ABELMANN, after the total extirpation of the pancreas, the fat of the food introduced is not absorbed at all, with the exception of milk, of which 28–53 per cent of the fat is absorbed. Other investigators have obtained different results, and HARLEY has observed a case where in a dog an absorption of only 4 per cent of the milk fat, or, on the complete exclusion of intestinal bacteria, even no absorption, took place. The conditions may vary in the different cases, and the behavior is not the same in different varieties of animals.

As shown by LOMBROSO, there exists an essential difference between the action of the extirpation of the gland, or a prevented flow of the secretion into the intestine. In the last case, as the experiments reported by NIEMANN show, no essential disturbance of the absorption takes place, while the total extirpation of the gland is followed by a marked disturbance (LOMBROSO[2]). This investigator is also of the opinion that the pancreas, independent of the external secretion in any way (by endocrinic bodies), influences the absorption of the foodstuffs and the activity of the pancreas enzymes in the intestine. In order to judge this view it would be of the greatest interest to know how the exclusion of the pancreatic juice from the intestine acts upon the other factors

[1] Müller, "Unters. über den Icterus," Zeitschr. f. klin. Med., **12**; Hédon and Ville, Arch. de Physiol. (5), **9**; Harley, Journ. of Physiol., **18**, Journ. of Pathol. and Bacteriol., 1895, and Proceed. Roy. Soc., **61**. In regard to the other authors see footnote 1, p. 532.

[2] Lombroso, see Bioch. Centralbl., **3**, 67 and 566, and **4**, 738; also Compt. rend. soc. biol., **57**; Hofmeister's Beiträge, 8, 11; Pflüger's Arch., **112**; and Arch. f. exp. Path. u. Pharm., **56** and **60**; Niemann, l. c.

of the digestion, such as upon the formation of the secretions and their activity. As to this we know at present very little, but the work of ZUNZ and MAYER (see page 532), indicates that such a reverse action is possible. Under these circumstances it is not possible to give LOMBROSO's views too great a prominence.

LOMBROSO has also found that after the extirpation of the pancreas in the dog, sometimes more fat is eliminated than was contained in the food; that this eliminated fat, which depends upon a fat secretion into the intestinal canal, has a different composition from the introduced fat, and that in these cases an absorption of fat also takes place. That some fat can be absorbed in animals even in the absence of the bile as well as pancreatic juice has been shown by the investigations of HÉDON and VILLE and CUNNINGHAM.[1]

The reason for the fact that the fat absorption is diminished in the absence of bile from the intestine must be sought for in the above-mentioned rôle of this fluid. It is more difficult to state why the absence of pancreatic juice causes a reduction in the absorption of fat. The most natural view is that the neutral fats are here less completely split, but this does not seem to be the case, because the non-absorbed fat of the feces consists, on the exclusion of bile and pancreatic juice (MINKOWSKI and ABELMANN, HARLEY, HÉDON and VILLE, DEUCHER), principally of free fatty acids. A still unknown change caused by gastric or intestinal lipase or by micro-organisms may produce a cleavage of the fat in these cases. The imperfect fat absorption after the extirpation of the pancreas can possibly be explained by the removal of a considerable part of the alkalies necessary for the formation of the emulsion and for the solution of the fatty acids, but as SANDMEYER found in dogs deprived of their pancreas, that the fat absorption was raised by giving chopped pancreas with the fat, this can hardly be a sufficient explanation. The reason for this is perhaps that after the extirpation of the pancreas the splitting of the fat is chiefly brought about by bacteria in those parts of the intestinal canal where the conditions for absorption are not favorable.

The soluble salts are also absorbed with the water. The proteins, which can dissolve a considerable quantity of salts, such as earthy phosphates which are otherwise insoluble in alkaline water, are of great importance in the absorption of such salts.

The soluble constituents of the digestive secretions can be absorbed like the other soluble substances and toxines, and ferments may also be absorbed, especially by a diseased change in the intestinal walls.

The occurrence of urobilin in urine attests the absorption of the bile-

[1] Hédon and Ville, l. c.; Cunningham, Journ. of Physiol., **22**.

constituents under physiological conditions despite the fact that the occurrence of very small traces of bile-acids in the urine is disputed. The absorption of bile-acids by the intestine seems to be positively proved by other observations. TAPPEINER[1] introduced a solution of bile-salts of a known concentration into an intestinal knot and after a time investigated the contents. He found that in the jejunum and the ileum, but not in the duodenum, an absorption of bile-acids took place, and further that of the two bile-acids only the glycocholic acid was absorbed in the jejunum. Further, SCHIFF long ago expressed the opinion that bile undergoes an intermediate circulation, in such wise that it is absorbed from the intestine, then carried to the liver by the blood, and lastly eliminated from the blood by this organ. Although this view has met with some opposition, still its correctness seems to be established by the researches of various investigators, and more recently by PREVOST and BINET, and specially by STADELMANN and his pupils.[2] After the introduction of foreign bile into the intestine of an animal, the foreign bile-acids appear again in the secreted bile.

How does the removal of large portions of the various parts of the intestine affect absorption? HARLEY[3] has been able to perform a partial extirpation of the large intestine and in another instance a complete extirpation. This last condition increased the feces considerably, especially because of the large increase in the water (five-fold). Fats and carbohydrates were absorbed just as completely as in the normal. The absorption of the proteins, on the contrary, was reduced to only 84 per cent as compared to 93–98 per cent in normal dogs. After extirpation, the feces sometimes did not contain any urobilin, or only traces thereof, while bile-pigments existed in large amounts.

ERLANGER and HEWLETT found that dogs from which 70–83 per cent of the total length of the jejunum and ileum had been removed, could be kept alive, like other animals, if only the food was not too rich in fat. When the food contained large amounts of fat then 25 per cent was evacuated by the feces as compared to 4–5 per cent in the normal animal. Under these same conditions the amount of nitrogen in the feces was increased to twice the normal amount. LONDON and STASSOW[4] found on resection of the ileum that the eliminated digestion and absorption were performed by the parts of the intestine higher

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[1] Wien. Sitzungsber., 77.

[2] Schiff, Pflüger's Arch., 8; Prevost and Binet, Compt. Rend., 106; Stadelmann, see footnote 1, p. 416.

[3] Proceed. Roy. Soc., 64.

[4] Erlanger and Hewlett, Amer. Journ. of Physiol. 6; London and Stassow, Zeitschr. f. physiol. Chem. 74, 349 (1911).

up; after resection of the jejunum the large intestine seems to have a compensating action.

After the exclusion of the colon in rabbits, BERGMANN and HULTGREN [1] could find no definite action upon the availability of the cellulose nor could any diminution in the utility of the other constituents of the food be observed. ZUNTZ and USTJANZEW [2] also found that the removal of the cæcum had no influence on the utilization of nitrogen; but in respect of other factors they arrive at different results. They found, namely, that the cæcum of the rodent is of great importance for the digestion of crude fiber and the pentosans. On feeding hay and wheat to rabbits after the removal of the cæcum, the digestion coefficient for crude fiber fell from 42.8 to 23.4–18.7 per cent, and for pentosans from 50 to 40–28.7 per cent.

The question as to the forces which are active in the intestine during absorption has not been satisfactorily answered. Attempts have been made to explain absorption as a filtration, due to a certain difference in the hydrostatic pressure between the intestinal contents and the blood. A sufficiently great difference in pressure does not seem to exist and besides this the absorbed solution on account of its composition cannot be considered as a filtrate from the intestinal contents. Diffusion processes without doubt play a much more important rôle. These attempt to keep the same concentration of all dissolved substances on both sides of the intestinal epithelium (in intestinal contents and in the blood). Such processes must be influenced, as mentioned in Chapter I on the osmotic pressure, to a high degree upon the permeability of the intestinal membrane for dissolved solids and for water. Nevertheless the diffusion stream does not give sufficient explanation for the absorption, as, according to COHNHEIM, [3] the result is different according to whether the intestine is alive or is dead and in general a streaming from the lumen of the intestine into the outside fluid is noticeable in the living intestine quite independent of the differences in concentration. How this streaming is brought about has not been explained.

Other investigators have suggested the question whether surface-tension forces (adsorption phenomenon) are active in absorption.[4] Still it has not been possible to bring the absorbability of a substance in simple relation to its influence on the surface-tension of the water.

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[1] Skand. Arch. f. Physiol., 14.

[2] Verhandl. d. physiol. Gesellsch. zu Berlin, 1904–1905.

[3] Zeitschr. f. physiol. Chem. 36–39.

[4] J. Traube, Bioch. Zeitschr. 24, 324 (1910) which also contains literature.

Under these circumstances and as it is not within the scope of this book to enter into details upon the numerous investigations as to the theory of absorption, we must refer to larger works [1] and to text-books on physiology for further information.

[1] See Höber, Physikalische Chemie der Zelle, Leipzig, 1906, Korányi and Richter, Physikalische Chemie u. Medizin. Leipzig 1907, Bd. 1, 295. I. Munk, Ergebnisse der Physiologie, I, Abt. 1; Hamburger, Osmotisher Druck und Ionenlehre, Bd. 2, Wiesbaden, 1904.

CHAPTER IX.

TISSUES OF THE CONNECTIVE SUBSTANCE.

I. THE CONNECTIVE TISSUES.

THE form-elements of the typical connective tissues are cells of various kinds, of a not very well-known chemical composition, and gelatin-yielding fibrils, which, like the cells, are imbedded in an interstitial or intercellular substance. The fibrils consist of *collagen*, the interstitial substance contains chiefly *mucoid* (*tendon-mucoid*), besides *serglobulin* and *seralbumin*, which occur in the parenchymatous fluid (LOEBISCH [1]).

The connective tissue also often contains fibers or formations consisting of *elastin*, sometimes in such great quantities that the connective tissue is transformed into elastic tissue. A third variety of fibers, the reticular fibers, also occurs, and according to SIEGFRIED these consist of *reticulin*.

If finely divided tendons are extracted in cold water or NaCl solutions, the protein bodies soluble in the nutritive fluid in addition to a little mucoid are dissolved. If the residue is extracted with half-saturated lime-water, then the mucoid is dissolved and may be precipitated from the filtered extract by adding an excess of acetic acid. The extracted residue contains the fibrils of the connective tissue together with the cells and the elastic substance.

The so-called tendon mucin is not true mucin, but a mucoid, which, as first shown by LEVENE and then by CUTTER and GIES, contains a part of its sulphur as an acid related to chondroitin-sulphuric acid. These mucoids, which, according to CUTTER and GIES, are mixtures of several glycoproteins, contain 2.2–2.33 per cent sulphur, as shown by the analyses of CHITTENDEN and GIES, as well as those of CUTTER and GIES. The quantity of sulphur split off as sulphuric acid was 1.33–1.62 per cent (CUTTER and GIES). VAN LIER [2] has prepared a substance at least closely related to tendon mucoid from the hard skin of man and certain animals. This mucoid yielded an ethereal sulphuric acid, a glucothionic acid with 1.58–3.03 per cent sulphur in the barium salt, and was variable in different animals. It gives the orcin reaction for glucuronic acid.

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[1] Zeitschr. f. physiol. Chem., **10**.

[2] Levene, *ibid.*, **31** and **39**; Cutter and Gies, Amer. Journ. of Physiol., **6**; Chittenden and Gies, Journ. of Exp. Med., **1**; van Lier, Zeitschr. f. physiol. Chem. **61**.

The fibrils of the connective tissue are elastic and swell slightly in water, somewhat more in dilute alkalies or in acetic acid. On the other hand, they shrink by the action of certain metallic salts, such as ferrous sulphate or mercuric chloride, and tannic acid, which form insoluble compounds with the collagen. Among these compounds, which prevent putrefaction of the collagen, that with tannic acid has been found of the greatest technical importance in the preparation of leather. In regard to the collagens, gelatins, elastins, and reticulins, see pages 116 to 121.

The tissues described under the names *mucous* or *gelatinous tissues* are characterized more by their physical than by their chemical properties, and have been but little studied. This much, however, is known, that the mucous or gelatinous tissues contain, at least in certain cases, as in the Acalephæ, no mucin.

The umbilical cord is the most accessible material for the investigation of the chemical constituents of the gelatinous tissues. The mucin occurring therein yields, according to VAN LIER, an ethereal sulphuric acid (glucothionic acid) like the tendon mucoid. C. TH. MÖRNER[1] has found a *mucoid* in the vitreous humor which contains 12.27 per cent nitrogen and 1.19 per cent sulphur.

Young connective tissue is richer in mucoid than old. HALLIBURTON[2] found an average of 7.66 p. m. mucoid in the skin of very young children and only 3.85 p. m. in the skin of adults. In so-called myxœdema, in which a re-formation of the connective tissue of the skin takes place, the quantity of mucoid is also increased.

The connective tissue and also the elastic tissue are richer in water and poorer in solids in young animals as compared with full-grown animals. This may be seen from the following analyses of the Achilles tendon (BUERGER and GIES) and of the ligamentum nuchæ (VANDEGRIFT and GIES[3]):

	Achilles tendon.		Ligament.	
	Calf.	Ox.	Calf.	Ox.
Water	675.1 p. m.	628.7 p. m.	651.0 p. m.	575.7 p. m.
Solids	324.9 "	371.3 "	394.0 "	424.3 "
Organic bodies	318.4 "	366.6 "	342.4 "	419.6 "
Inorganic bodies	6.1 "	4.7 "	6.6 "	4.7 "
Fat	.....	10.4 "	.....	11.2 "
Proteid	.....	2.2 "	.....	6.16 "
Mucoid	.....	12.83 "	.....	5.25 "
Elastin	.....	16.33 "	.....	316.70 "
Collagen	.....	315.88 "	.....	72.30 "
Extractives, etc.	.....	8.96 "	.....	7.99 "

[1] Zeitschr. f. physiol. Chem., 18, 250.

[2] Mucin in Myxœdema: Further Analyses. King's College Collected Papers No. 1, 1893.

[3] Buerger and Gies, Amer. Journ. of Physiol., 6; Vandegrift and Gies, *ibid*, 5.

In regard to the mineral bodies it must be remarked that according to the determinations of H. SCHULZ the connective tissue is rich in silicic acid. The greatest amount was found by him, in the crystalline lens of the ox, namely, 0.5814 gram per kilo of dried substance. In man he found 0.0637 gram in the tendons, 0.1064 gram in the fascia, and 0.244 gram in Wharton's jelly for every kilo of dried substance. The quantity of silicic acid is higher in the young than in the old; in man it is highest in the embryonic connective tissue of the umbilical cord. In the last-named substance SCHULZ also found 0.403 gram Fe_2O_3, 0.693 gram MgO, 3.297 grams CaO, and 3.794 grams P_2O_5 for every kilo of dried substance. The report of SCHULZ on the quantity of silicic acid does not correspond with the investigations of FRAUENBERGER[1] who found, in Wharton's jelly, only a fraction of the quantity of silicic acid that SCHULZ gives.

II. CARTILAGE.

Cartilaginous tissues consist of cells and an original hyaline matrix, which, however, may become changed in such wise that there appears in it a network of elastic fibers or connective-tissue fibrils.

Those cells that offer great resistance to the action of alkalies and acids have not been carefully studied. According to earlier opinions the matrix was considered as consisting of a body analogous to collagen, so-called *chondrigen*. The investigations of MOROCHOWETZ and others, but especially those of C. MÖRNER,[2] have shown that the matrix of the cartilage consists of a mixture of collagen with other bodies.

The tracheal, thyroideal, cricoidal, and arytenoidal cartilages of full-grown cattle contain, according to MÖRNER, four constituents in the matrix, namely, *chondromucoid*, *chondroitin-sulphuric acid*, *collagen*, and the *albumoid*.

Chondromucoid. This body, according to C. MÖRNER, has the composition C 47.30, H 6.42, N 12.58, S 2.42, O 31.28 per cent. Sulphur is in part loosely combined and may be split off by the action of alkalies, and a part separates as sulphuric acid when boiled with hydrochloric acid. Chondromucoid is decomposed by dilute alkalies and yields alkali albuminate, peptone substances, chondroitin-sulphuric acid, alkali sulphides, and some alkali sulphates. On boiling with acids it yields acid albuminate, peptone substances, chondroitin-sulphuric acid, and on

[1] Schulz, Pflüger's Arch, 84 and 89, 131 and 144; Frauenberger, Zeitschr. f. physiol. Chem., 57.

[2] Morochowetz, Verhandl., d. naturh. med. Vereins zu Heidelberg, 1, Heft 5; Mörner, Skand. Arch. f. Physiol., 1.

account of the further decomposition of this last body, sulphuric acid and a reducing substance are formed.

Chondromucoid is a white, amorphous, acid-reacting powder which is insoluble in water, but dissolves easily on the addition of a little alkali. This solution is precipitated by acetic acid in great excess and by small quantities of mineral acids. The precipitation may be retarded by neutral salts or by chondroitin-sulphuric acid. The solution containing NaCl and acidified with HCl is not precipitated by potassium ferrocyanide. Precipitants for chondromucoid are alum, ferric chloride, sugar of lead, or basic lead acetate. Chondromucoid is not precipitated by tannic acid, and it may by its presence prevent the precipitation of gelatin by this acid. It gives the usual color reactions for proteins, namely, with nitric acid, with copper sulphate and alkali, with MILLON'S and ADAMKIEWICZ-HOPKINS' reagents.

Chondroitin-sulphuric Acid, CHONDROITIC ACID. This acid, which was first prepared pure, from cartilage, by C. MÖRNER and identified by him as an ethereal sulphuric acid, occurs, according to MÖRNER, in all varieties of cartilage and also in the tunica intima of the aorta and as traces in the bone substance. K. MÖRNER[1] has also found it in the ox-kidney and in human urine as a regular constituent. Its occurrence in amyloid, as mentioned on page 173, has been disputed by HANSSEN. In the opinion of LEVENE,[2] the glucothionic acid which is prepared from tendon mucoid, and which gives the orcin reaction for glucuronic acid, and yields furfurol on distillation with hydrochloric acid, is not identical with the chondroitin-sulphuric acid, but is probably related thereto.

Chondroitin-sulphuric acid has the formula $C_{18}H_{27}NSO_{17}$, according to SCHMIEDEBERG.[3] As primary products this acid yields, on cleavage, sulphuric acid and a nitrogenous substance, *chondroitin*, according to the following equation:

$$C_{18}H_{27}NSO_{17}+H_2O=H_2SO_4+C_{18}H_{27}NO_{14}.$$

Chondroitin, which is similar to gum arabic, and which is a monobasic acid, yields acetic acid and a new nitrogenous substance, *chondrosin*, as cleavage products, on decomposition with dilute mineral acids:

$$C_{18}H_{27}NO_{14}+3H_2O=3C_2H_4O_2+C_{12}H_{21}NO_{11}.$$

Chondrosin, which is also a gummy substance soluble in water, is a monobasic acid and reduces copper oxide in alkaline solutions even

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[1] C. Mörner, l. c., and Zeitschr. f. physiol. Chem., **20** and **23**; K. Mörner, Skand. Arch. f. Physiol., **6**.

[2] Zeitschr. f. physiol. Chem., **39**.

[3] Arch. f. exp. Path. u. Pharm., **28**.

more strongly than glucose. It is dextrogyrate, and represents the reducing substance obtained by previous investigators in an impure form on boiling cartilage with an acid. The products obtained on decomposing chondrosin with barium hydroxide tend to show, according to SCHMIEDEBERG, that chondrosin contains the atomic groups of glucuronic acid and glucosamine. This assumption does not seem to have sufficient foundation. According to ORGLER and NEUBERG, chondrosin does not give the orcin test nor does it yield furfurol. They claim that on cleavage with baryta it yields, besides a carbohydrate complex which has not been studied, an oxyamino-acid having the formula $C_6H_{13}O_6N$, a hexosamine acid or tetraoxyaminocaproic acid. In opposition to this S. FRÄNKEL has found that the chondrosin gives the orcin as well as the phloroglucin test with hydrochloric acid, and he has prepared an acid with the formula $C_6H_{11}NO_6$, which he calls *aminoglucuronic acid*, which gives the above tests and also reduces. Among other investigators, PONS and KONDO[1] have also found that chondroitin-sulphuric acid gives the orcin test and yields furfurol, according to PONS 6.6–6.9 per cent. The chondrosin obtained after boiling with acid and distilling off the furfurol does not, according to PONS, give furfurol, which agrees with ORGLER and NEUBERG'S statement. From the hydrolytic products of chondroitin-sulphuric acid with hydrochloric acid, PONS obtained with phenylhydrazin a crystalline substance melting at 143° C.

Chondroitin-sulphuric acid appears as a white amorphous powder which dissolves very easily in water, forming an acid solution and, when sufficiently concentrated, a sticky liquid similar to a solution of gum arabic. Nearly all of its salts are soluble in water. The neutralized solution is precipitated by stannous chloride, basic lead acetate, neutral ferric chloride, and by alcohol in the presence of a little neutral salt. The solution, on the other hand, is not precipitated by acetic acid, tannic acid, potassium ferrocyanide and acetic acid, sugar of lead, mercuric chloride, or silver nitrate. Acidified solutions of alkali chondroitin-sulphates cause a precipitation when added to solutions of gelatin or proteid.

The preparation of chondromucoid, and its separation from chondroitin-sulphuric acid can be accomplished after the method of C. MÖRNER, but for details we refer to the original work.

The pre-existing chondroitin-sulphuric acid, or that formed by the decomposition of chondromucoid, is obtained by lixiviating the cartilage with a 5-per cent caustic-alkali solution. The alkali albuminate formed by the decomposition of the chondromucoid can be removed from the solution by neutralization, then the peptone precipitated by tannic acid,

[1] Orgler and Neuberg, Zeitschr. f. physiol. Chem., **37**; Fränkel, Annal. d. Chem. u. Pharm., **351**; Pons. Arch. intern. de Physiol., 8 (1909); Kondo, Bioch. Zeitschr., **26**.

the excess of this acid removed with sugar of lead, and the lead removed from the filtrate by H_2S. If further purification is necessary, the acid is precipitated with alcohol, the precipitate dissolved in water, this solution dialyzed and precipitated again with alcohol—this solution in water and precipitation with alcohol being repeated a few times—and lastly the acid is treated with alcohol and ether. Other methods for the preparation of the acid (from the septum narium of the pig) have been suggested by SCHMIEDEBERG and KONDO.

The *collagen* of the cartilage gives, according to C. MÖRNER, a gelatin which contains only 16.4 per cent N, and which can hardly be considered identical with ordinary gelatin.

In the above-mentioned cartilages of full-grown animals the chondroitin-sulphuric acid and chondromucoid, perhaps also the collagen, are found surrounding the cells as round balls or lumps. These balls (MÖRNER'S *chondrin-balls*), which give a blue color with methyl-violet, lie in the meshes of a trabecular structure, which is colored when brought in contact with tropæolin.

The *albumoid* is a nitrogenized body which contains loosely combined sulphur. It is soluble with difficulty in acids and alkalies and resembles keratin in many respects, but differs from it by being soluble in gastric juice. In other respects it resembles elastin, but differs from this substance in containing sulphur. This albumoid gives the color reactions of the protein bodies.

Cartilage gelatin and the albumoid may be prepared according to the folowing method of MÖRNER: First remove the chondromucoid and chondroitin-sulphuric acid by extraction with dilute caustic potash (0.2–0.5 per cent), remove the alkali from the remaining cartilage by water, and then boil with water in a PAPIN'S digester. The collagen passes into solution as gelatin, while the albumoid remains undissolved (contaminated by the cartilage-cells). The gelatin may be purified by precipitating with sodium sulphate, which must be added to saturation in the faintly acidified solution, redissolving the precipitate in water, dialyzing well, and precipitating with alcohol.

In MÖRNER'S experience no albumoid is found in young cartilage, but only the three first-mentioned constituents. Nevertheless, the young cartilage contains about the same amounts of nitrogen and mineral substances as the old. The cartilage of the ray (*Raja batis* LIN.), which has been investigated by LÖNNBERG,[1] contains no albumoid and only a little chondromucoid, but a large proportion of chondroitin-sulphuric acid and collagen.

According to PFLÜGER and HÄNDEL,[2] glycogen occurs to a slight

[1] Maly's Jahresber., **19**, 325.

[2] Pflüger in Pflüger's Arch., **92**; Händel, *ibid.*

extent in all matrices, and of these it is richest in the cartilage. Tendons, ligamentum nuchæ, and cartilage of the ox contained 0.06, 0.07, and 2.17 p. m. glycogen respectively (HÄNDEL).

HOPPE-SEYLER found in fresh human rib-cartilage 676.7 p. m. water, 301.3 p. m. organic, and 22 p. m. inorganic substance, and in the cartilage of the knee-joint 735.9 p. m. water, 248.7 p. m. organic, and 15.4 p. m. inorganic substance. PICKARDT found 402–574 p. m. water and 72.86 p. m. ash (no iron) in the laryngeal cartilage of oxen. The ash of cartilage contains considerable amounts (even 800 p. m.) of alkali sulphate, which probably does not exist originally as such, but is produced in great part by the incineration of the chondroitin-sulphuric acid and the chondromucoid. The analyses of the ash of cartilage therefore cannot give a correct idea of the quantity of mineral bodies existing in this substance. The cartilage is richest in sodium of all the tissues of the body, and according to BUNGE [1] the amount of Na and Cl is greatest in young animals. In 1000 parts of cartilage dried at 120° C., BUNGE found 91.26 parts Na_2O in the shark, 33.98 in the ox embryo, 32.45 in a fourteen-day-old calf, and 26.4 in a ten-weeks-old calf.

Ochronose is the brown to black coloration of the cartilage which sometimes occurs, and which has also been observed in several cases of alcaptonuria (see Chapter XIV) or after lengthy treatment with carbolic acid bandages (POULSEN, ADLER [2]). The nature of these melanine-like pigments is unknown.

The Cornea. The corneal tissue, which, in a chemical sense, is considered by many investigators to be related to cartilage, contains traces of proteid and a *collagen* as chief constituent, which C. MÖRNER [3] claims contains 16.95 per cent N. According to him it also contains a *mucoid* which has the composition C 50.16, H 6.97, N 12.79, and S 2.07 per cent. On boiling with dilute mineral acid this mucoid yields a reducing substance. The globulins found by other investigators in the cornea are not derived from the matrix, according to MÖRNER, but from the layer of epithelium. MÖRNER believes that DESCEMET'S membrane consists of *membranin* (page 171), which contains 14.77 per cent N and 0.90 per cent S.

In the cornea of oxen HIS [4] found 758.3 p. m. water, 203.8 p. m. gelatin-forming substance, 28.4 p. m. other organic substance, besides 8.1 p. m. soluble and 1.1 p. m. insoluble salts.

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[1] Hoppe-Seyler, cited from Kühne's Lehrbuch d. physiol. Chem., 387; Pickardt, Centralbl. f. Physiol., 6, 735; Bunge, Zeitschr. f. physiol. Chem., 28.

[2] See Maly's Jahresb., 40, 424, Adler, Zeitschr. f. Krebsforschung, 11.

[3] Zeitschr. f. physiol. Chem., 18.

[4] Cited from Gamgee, Physiol. Chem., 1880, 451.

III. BONE.

The bony structure proper, when free from other formations occurring in bones, such as marrow, nerves, and blood-vessels, consists of cells and a matrix.

The *cells* have not been closely studied in regard to their chemical constitution. On boiling with water they yield no gelatin. They contain no keratin, which usually should not be present in the bony structure (HERBERT SMITH [1]).

The *matrix* of the bony structure contains two chief constituents, namely, an organic substance, and the so-called *bone-earths*, lime-salts, inclosed in or combined with it. If bones are treated with dilute hydrochloric acid at the ordinary temperature, the lime-salts are dissolved and the organic substance remains as an elastic mass, preserving the shape of the bone.

The organic matrix consists chiefly of *ossein*, which is generally considered as identical with the collagen of the connective tissue. It also contains, as HAWK and GIES [2] have shown, *mucoid* and *albuminoid*. After the removal of the lime-salts by hydrochloric acid of 2–5 p. m. these experimenters were able to extract the mucoid by one-half saturated lime-water, and to precipitate it with 2 p. m. hydrochloric acid. After the removal of the osseomucoid and collagen (by boiling with water) they obtained the albuminoid as an insoluble residue.

The osseomucoid on boiling with hydrochloric acid yielded a reducing substance and sulphuric acid; 1.11 per cent sulphur appearing in this form. The osseomucoid stands close to the chondro- and tendon mucoid in elementary composition, as may be seen from the following analyses:

	C	H	N	S	O	
Osseomucoid.....	47.43	6.63	12.22	2.32	31.40	(HAWK and GIES)
Chondromucoid....	47.30	6.42	12.58	2.42	31.28	(C. MÖRNER)
Tendon mucoid....	48.76	6.53	11.75	2.33	30.60	(CHITTENDEN and GIES)
Corneal mucoid....	50.16	6.97	12.79	2.07	28.01	(C. MÖRNER)

The osseoalbuminoid is insoluble in 2 p. m. hydrochloric acid, and in 5 p. m. Na_2CO_3, but dissolves in 10 per cent KOH with the formation of albuminates. The composition of chondro- and osseoalbuminoid is as follows:

	C	H	N	S	O	
Osseoalbuminoid.....	50.16	7.03	16.17	1.18	25.46	HAWK and GIES
Chondroalbuminoid...	50.46	7.05	14.95	1.86	25.68	

[1] Zeitschr. f. Biologie, 19.

[2] Amer. Journ. of Physiol., 5 and 7.

The inorganic constituents of the bony structure, the so-called *bone-earths*, which, after the complete calcination of the organic substance, remain as a white brittle mass, consist chiefly of calcium and phosphoric acid, but also contain carbonic acid and, in smaller amounts, magnesium, chlorine, and fluorine. Iron, which has been found in bone-ash, does not seem to belong exactly to the bony substance, but to the nutritive fluids or to the other constituents of bones. The traces of sulphate occurring in the bone-ash are derived, according to MÖRNER, from the chondroitin-sulphuric acid. According to GABRIEL, potassium and sodium are essential constituents of bone-earth, and this has been substantiated by ARON [1].

The opinions of investigators differ slightly as to the manner in which the mineral bodies of the bony structure are combined with each other. Chlorine is present in the same form as in apatite $3(Ca_3P_2O_8)CaCl_2$. If we eliminate the magnesium, the chlorine, and the fluorine, the last, GABRIEL claims, occurring only as traces, the remaining mineral bodies form the combination $3(Ca_3P_2O_8)CaCO_3$. In his opinion the simplest expression for the composition of the ash of bones and teeth is $(Ca_3(PO_4)_2 + Ca_5HP_3O_{13} + Aq)$, in which 2–3 per cent of the lime is replaced by magnesia, potash, and soda, and 4–6 per cent of the phosphoric acid by carbonic acid, chlorine, and fluorine. Recently, on the contrary, GASSMANN has given important reasons for the following complex combination in WERNER'S [2] sense.

$$\left[Ca \left(\begin{matrix} OPO_3Ca \\ > Ca \\ OPO_3Ca \end{matrix} \right) 3 \right] CO_3$$

Analyses of bone-earths have shown that the mineral constituents exist in rather constant proportions, which are nearly the same in different animals. As an example of the composition of bone-earth we here give the analyses of ZALESKY.[3] The figures represent parts per thousand:

	Man.	Ox.	Tortoise.	Guinea-pig.
Calcium phosphate, $Ca_3P_2O_8$	838.9	860.9	859.8	873.8
Magnesium phosphate, $Mg_3P_2O_8$	10.4	10.2	13.6	10.5
Calcium combined with CO_2, Fl, and Cl	76.5	73.6	63.2	70.3
CO_2	57.3	62.0	52.7	.....
Chlorine	1.8	2.0	.....	1.3
Fluorine[4]	2.3	3.0	2.0	.....

[1] Mörner, Zeitschr. f. physiol. Chem., **23**; Gabriel, *ibid.*, **18**, which also contains the pertinent literature; Aron, Pflüger's Arch., **106**.

[2] Gassmann, Zeitschr. f. physiol. Chem. **70** and **83**; Werner, Ber. d. d. Chem. Gesellsch., **40**.

[3] Hoppe-Seyler, Med.-chem. Untersuch., p. 19.

[4] The reports as to the quantity of fluorine disagree; see Harms, Zeistchr. f. Biologie, **38**; Jodlbauer, *ibid.*, **41**.

Some of the CO_2 is always lost on calcining, so that the bone-ash does not contain the entire CO_2 of the bony substance.

GAUTIER and CLAUSMANN [1] have determined the fluorine in various organs and tissues. In man the diaphysis end of the femur had 0.495 p. m. fluorine, and the epiphysis end 0.119 p. m. fluorine. In children the diaphysis end of the long bones contained 0.156 p. m. fluorine and the epiphysis end 0.037 p. m. A similar difference also occurs in animals. Cartilage of man with 0.014 p. m. fluorine and tendons (calf) with 0.0035 p. m. fluorine, are much poorer in fluorine than the bones. The dentin (dog) contains 0.56 p. m. fluorine and the enamel (of a young dog) contained 1.66. p. m. fluorine, all results obtained from the fresh substance.

AD. CARNOT [2] found the following composition for the bone-ash of man, ox, and elephant:

	Man.		Ox.	Elephant.
	Femur (body).	Femur (head).	Femur	Femur.
Calcium phosphate	874.5	878.7	857.2	900.3
Magnesium phosphate	15.7	17.5	15.3	19.6
Calcium fluoride	3.5	3.7	4.5	4.7
Calcium chloride	2.3	3.0	3.0	2.0
Calcium carbonate	101.8	92.3	119.6	72.7
Iron oxide	1.0	1.3	1.3	1.5

The quantity of organic substance in the bones, calculated from the loss of weight in burning, varies between 300 and 520 p. m. This variation may in part be explained by the difficulty in obtaining the bony substance entirely free from water, and partly by the very variable amount of blood-vessels, nerves, marrow, and the like in different bones. The unequal amounts of organic substance found in the compact and in the spongy parts of the same bone, as well as in bones at different periods of development in the same animal, probably depend upon the varying quantities of these above-mentioned tissues. *Dentin*, which is comparatively pure bony structure, contains only 260–280 p. m. organic substance, and HOPPE-SEYLER [3] therefore thinks it probable that perfectly pure bony substance has a constant composition and contains only about 250 p. m. organic substance. The question whether these substances are chemically combined with the bone-earths or only intimately mixed has not been decided.

The nutritive fluids which circulate through the bones have not been isolated and we only know that they contain some protein and some NaCl and alkali sulphate.

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[1] Compt. Rend., 156.
[2] *Ibid.*, 114.
[3] Physiol. Chem., 102–104.

Bone Marrow. We differentiate between the red and yellow marrow, to which also belongs the gelatinous marrow, poor in fat, found in fat atrophy and in old age. The difference between the first two-mentioned kinds of marrow lies, essentially, in the fact that the red marrow contains a greater quantity of erythrocytes besides a higher content of protein and less fat. The fat of the yellow marrow is, according to NERKING,[1] richer in oleic acid and poorer in solid fats than the fat of the red marrow. Besides the fat, lecithin also occurs in the bone-marrow and this varies in amount in different animals and at various ages, as mentioned on page 244. The protein consists of a globulin coagulating at 47–50° C. (FORREST) and a nucleoprotein with 1.6 per cent phosphorus (HALLIBURTON [2]) besides fibrinogen (P. MÜLLER [3]), traces of albumin and proteose. In the extractives are found lactic acid, inosite, hypoxanthine, cholesterine and bodies of an unknown kind. The quantitative composition of both kinds of marrow varies considerably with the fat content, and the reports of the different investigators are correspondingly discrepant (NERKING, HUTCHINSON and MACLEOD [4]).

The diverse quantitative composition of the various bones of the skeleton depends probably on the varying quantities of other tissues, such as marrow, blood-vessels, etc., which they contain. The same reason explains, to all appearances, the larger quantity of organic substance in the spongy part of the bones as compared with the more compact parts. SCHRODT [5] has made comparative analyses of different parts of the skeleton of the same animal (dog) and has found an essential difference. The quantity of water in the fresh bones varies between 138 and 443 p. m. The bones of the extremities and the skull contain 138–222, the vertebræ 168–443, and the ribs 324–356 p. m. water. The quantity of fat varies between 13 and 269 p. m. The largest amount of fat, 256–269 p. m., is found in the long tubular bones, while only 13–175 p. m. fat is found in the small short bones. The quantity of organic substance, calculated from fresh bones, was 150–300 p. m., and the quantity of mineral substances 290–563 p. m. Contrary to the general supposition the greatest amount of bone-earths was not found in the femur, but in the first three cervical vertebræ. In birds the tubular bones are richer in mineral substances than the flat bones (DÜRING), and the greatest quantity of mineral bodies has been found in the humerus (HILLER, DÜRING [6]).

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[1] Bioch. Zeitschr., **10**.

[2] Forrest, Journ. of Physiol., **17**; Halliburton, *ibid.*, **18**.

[3] See footnote 1, p. 253.

[4] Nerking, l. c.; Hutchinson and Macleod, Journ. of Anat. and Physiol., **26**.

[5] Cited from Maly's Jahresber., **6**.

[6] Hiller, cited from Maly's Jahresber., **14**; Düring, Zeitschr. f. physiol. Chem., **23**.

We do not possess trustworthy information in regard to the composition of bones at different ages. The analyses by E. VOIT of bones of dogs, and by BRUBACHER of bones of children, apparently indicate that the skeleton becomes poorer in water and richer in ash with increase in age. GRAFFENBERGER [1] has found in rabbits, 6½–7½ years old, that the bones contained only 140–170 p. m. water, while the bones of the full-grown rabbit 2–4 years old contained 200–240 p. m. The bones of old rabbits contain more carbon dioxide and less calcium phosphate.

The composition of bones of animals of different species is but little known. The bones of birds contain, as a rule, somewhat more water than those of mammalia, and the bones of fishes contain the largest quantity of water. The bones of fishes and amphibians contain a greater amount of organic substance. The bones of pachyderms and cetaceans contain a large proportion of calcium carbonate; those of granivorous birds always contain silicic acid. The bone-ash of amphibians and fishes contains sodium sulphate. The bones of fishes seem to contain more soluble salts than the bones of other animals.

A great many experiments have been made to determine the exchange of material in the bones—for instance, with food rich in lime and with food deficient in lime—but the results have always been doubtful or contradictory. The attempts to substitute other alkaline earths or alumina for the lime of the bones have also given conflicting results.[2] On feeding sufficient calcium and phosphorus in the food ARON [3] found, by strongly reducing the sodium and at the same time giving a large amount of potassium, that the development of the bones was below normal. On the administration of madder, the bones of the animal are found to be colored red after a few days or weeks; but these experiments have not led to any positive conclusion in regard to the growth or metabolism in the bones.

Under pathological conditions, as in rachitis and softening of the bones, an ossein has been found which does not give any typical gelatin on boiling with water. This finding is still uncertain as otherwise pathological conditions seem to affect chiefly the quantitative composition of the bones, and especially the relation between the organic and the inorganic substance. In rachitis the bones are poorer in solids and these are poorer in mineral substances than under normal conditions. Attempts have been made to produce rachitis in animals by the use of food deficient in lime. From experiments on fully developed animals opposing results have been obtained. In young, undeveloped animals

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[1] Voit, Zeitschr. f. Biologie, 16; Brubacher, *ibid.*, 27; Graffenberger in Maly's Jahresber., 21.

[2] See H. Weiske, Zeitschr. f. Biologie, 31, and W. Stoeltzner, Pflüger's Arch., 122, and H. Stoeltzner, Bioch. Zeitschr., 12.

[3] Pflüger's Arch., 106.

ERWIN VOIT, ARON and SEBAUER and others[1] produced, by lack of lime-salts, a change similar to rachitis. In full-grown animals the bones were changed after a long time because of the lack of lime-salts in the food, but did not become soft, only thinner (osteoporosis). The attempts to remove the lime-salts from the bones by the addition of lactic acid to the food have led to no positive results (HEITZMANN, HEISS, BAGINSKY[2]). WEISKE, on the contrary, has shown, by administering dilute sulphuric acid or monosodium phosphate with the food (presupposing that the food gave no alkaline ash) to sheep and rabbits, that the quantity of mineral bodies in the bones might be diminished. On feeding continuously for a long time with a food which yielded an acid ash (cereal grains), WEISKE observed a diminution in the mineral substances of the bones in full-grown herbivora.[3] A few investigators are of the opinion that in rachitis, as in osteomalacia, in which disease the calcium content of the bones is also diminished, a solution of the lime-salts by means of lactic acid takes place. This was suggested by the fact that O. WEBER and C. SCHMIDT[4] found lactic acid in the cyst-like, altered bony substance in osteomalacia.

Well-known investigators have disputed the possibility of the lime-salts being washed from the bones in osteomalacia by means of lactic acid. They have given special prominence to the fact that the lime-salts held in solution by the lactic acid must be deposited on neutralization of the acid by the alkaline blood. This objection is not very important, as the alkaline blood-serum has the property to a high degree of holding earthy phosphates in solution, which fact has been recently shown by HOFMEISTER. The investigations of LEVY contradict the claim as to the solution of the lime-salts by lactic acid in osteomalacia. He found that the normal relation $6PO_4:10Ca$ is retained in all parts of the bones in osteomalacia, which would not be the case if the bone-earths were dissolved by an acid. The decrease in phosphate occurs in the same quantitative relation as the carbonate, and according to LEVY, in osteomalacia the exhaustion of the bone takes place by a decalcification in which one molecule of phosphate-carbonate after the other is removed. This does not agree with the findings of MCCRUDDEN[5] who

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[1] Zeitschr. f. Biologie, **16**; Aron and Sebauer, Bioch. Zeitschr., **8**; A. Baginsky, Arch. f. (Anat. u.) Physiol., 1881.

[2] Heitzmann, Maly's Jahresber., **3**, 229; Heiss, Zeitschr. f. Biologie, **12**; Baginsky; Virchow's Arch., **87**.

[3] See Maly's Jahresber., **22**; also Weiske, Zeitschr. f. physiol. Chem., **20**, and Zeitschr. f. Biologie, **31**.

[4] Cited from v. Gorup-Besanez, Lehrb. de. physiol. Chem., 4. Aufl.

[5] Hofmeister, Ergebn. d. Physiol. **10**; Levy, Zeitschr. f. physiol. Chem. **19**; McCrudden, Journ. of biol. Chem., **7**.

found a changed relation between the Ca and phosphoric acid in osteomalacia.

Rachitic bones are always poorer in mineral substances than normal bones. The relation between Ca_3PO_4 and CO_2 was found by GASSMANN to be the same as in normal bones while he found a pathological increase in the magnesium. The organic substance was found in rachitis to be relatively as well as absolutely increased, at least in certain cases (GASSMANN). The statements differ in regard to the water content. According to BRUBACHER this is larger while according to GASSMANN it is 10 p. m. smaller than in normal bones. In opposition to rachitis, osteomalacia is often characterized by the considerable amount of fat in the bones, 230–290 p. m., but as a rule the composition varies so much that the analyses are of little value. In a case of osteomalacia, CHABRIE [1] found a larger quantity of magnesium than calcium in a bone. The ash contained 417 p. m. phosphoric acid, 222 p. m. lime, 269 p. m. magnesia, and 86 p. m. carbon dioxide. MCCRUDDEN found more magnesium than calcium; other investigators have on the contrary found more calcium than magnesium.

The **tooth-structure** is closely related, from a chemical standpoint, to the bony structure.

Of the three chief constituents of the teeth—dentin, enamel, and cement—the *cement* is to be considered as true bony structure, and as such has already been discussed to some extent. *Dentin* has the same composition as the bony structure, but contains somewhat less water. The organic substance yields gelatin on boiling; but the dental tubes are not dissolved, therefore they cannot consist of collagen. In dentin 260–280 p. m. organic substance has been found. *Enamel* is an epithelium formation containing a large proportion of lime-salts. Corresponding to its character and origin, the organic substance of the enamel does not yield any gelatin. Completely developed enamel contains the least water, the greatest quantity of mineral substances, and is the hardest of all the tissues of the body. In full-grown animals it contains hardly any water, and the quantity of organic substance amounts to only 20–40–68 p. m. The relative amounts of calcium and phosphoric acid are shown by the analyses of HOPPE-SEYLER to be about the same as in bone-earths. The quantity of chlorine according to him is remarkably high, 0.3–0.5 per cent, while BERTZ [2] found that the ash of enamel was free from chlorine and that dentin was very poor in chlorine.

CARNOT,[3] who has investigated the dentin from elephants, has found 4.3 p. m. calcium fluoride in the ash. In ivory he found only 2 p. m. Dentin from elephants is rich in magnesium phosphate, which is still more abundant in ivory.

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[1] Gassmann, Zeitschr. f. physiol. Chem. **70**; Brubacher, Zeitschr. f. biol. **27**. See also Cappezzuoli, Bioch. Zeitschr. **16**; Chabrié, Les phénomènes chim. de l'ossification, Paris, 1895, 65.

[2] See Maly's Jahresber., **30**.

[3] Compt. Rend., **114**.

GABRIEL found that the quantity of fluorine is very small and amounts to 1 p. m. in ox-teeth. It is no greater in the teeth and enamel than in the bones.[1] The same investigator found that the amount of phosphates is strikingly small in the enamel, and in the teeth considerable lime is replaced by magnesia. This coincides with BERTZ's findings, that dentin contains twice as much magnesia as the enamel.

According to GASSMANN,[2] the teeth among themselves have different composition, and in man the wisdom teeth are poorer in organic substance and richer in lime than the canine teeth. The great tendency of the first to caries is probably explained by this fact. The reason for the degeneration of the teeth is considered by C. RÖSE[3] to be a lack of earthy salts, and according to him one finds the best teeth in localities where the drinking water has high permanent hardness.

IV. THE FATTY TISSUE.

The membranes of the fat-cells withstand the action of alcohol and ether. They are not dissolved by acetic acid or by dilute mineral acids, but are dissolved by artificial gastric juice. They may possibly consist of a substance closely related to elastin. The fat-cells contain, besides fat, a yellow pigment which in emaciation does not disappear so rapidly as the fat; and this is the reason that the subcutaneous cellular tissue of an emaciated corpse has a dark orange-red color. The cells deficient in, or nearly free from fat, which remain after the complete disappearance of the latter, seem to have an albuminous protoplasm rich in water. Adipose tissue is rich in a fat-splitting enzyme and in catalases.

The less water the fatty tissue contains the richer it is in fat. SCHULZE and REINECKE[4] found in 1000 parts:

	Water.	Membrane.	Fat.
Fatty tissue of oxen	99.7	16.6	883.7
Fatty tissue of sheep	104.8	16.4	878.8
Fatty tissue of pigs	64.4	13.6	922.0

The fat contained in the fat-cells consists mainly of triglycerides of stearic, palmitic, and oleic acids. Besides these, especially in the less solid kinds of fats, there are glycerides of other fatty acids (see Chapter IV). In all animal fats there are besides these, as FR. HOFMANN[5] has

[1] See footnote 4, p. 552.
[2] Zeitschr. f. physiol. Chem., 55.
[3] Deutsch. Monatsh. f. Zahnheilk., 1908.
[4] Annal. d. Chem. u. Pharm., 142.
[5] Ludwig-Festschrift, 1874, Leipzig.

shown, also free, non-volatile fatty acids, although in very small amounts.

Human fat is relatively rich in olein, the quantity in the subcutaneous fatty tissue being 70–80 per cent or more.[1] In new-born infants it is poorer in oleic acid than in adults (KNÖPFELMACHER, SIEGERT, JAECKLE); the quantity of olein increases until the end of the first year, when it is about the same as in adults. The composition of the fat in man as well as in different individuals of the same species of animals is rather variable, a fact which is probably dependent upon the food. According to the researches of HENRIQUES and HANSEN the fat of the subcutaneous fatty tissue is richer in olein than that of the internal organs; this has also been observed by LEICK and WINKLER.[2] In animals with a thick subcutaneous fat deposit the outer layers, according to HENRIQUES and HANSEN, are richer in olein than the inner layers. The fat of cold-blooded animals is especially rich in olein, The fat of domestic animals has, according to AMTHOR and ZINK, a less oily consistency and a lower iodine and acetyl equivalent than the corresponding fat of wild animals. Under pathological conditions the fat may have a markedly pronounced variation. The fat of lipoma seems, from JAECKLE's experience, to be poorer in lecithin than other fats.

The fat stored up in the organs and tissues can be changed somewhat by the composition of the fat of the food, still, according to ABDERHALDEN and BRAHM,[3] the fat actually occurring in the cells (with the exception of the real fat cells) is not dependent in its composition upon the kind of food fat taken.

The properties of fats in general, and the three most important varieties of fat in particular, have been considered in a previous chapter, hence the formation of the adipose tissue is of chief interest at this time.

The formation of fat in the organism may occur in various ways. The fat of the animal body may consist partly of fat absorbed from the food and deposited in the tissues, and partly of fat formed in the organism from other bodies, such as proteins (?) or carbohydrates.

That the fat from the food which is absorbed in the intestinal canal may be retained by the tissues has been shown in several ways. RADZIEJEWSKI, LEBEDEFF, and MUNK have fed dogs with various fats, such as linseed-oil, mutton-tallow, and rape-seed-oil, and have afterward

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[1] See Jaeckle, Zeitschr. f. physiol. Chem., **36** (literature).

[2] Knöpfelmacher, Jahrbuch f. Kinderheilkunde (N. F.), **45** (older literature); Siegert, Hofmeister's Beiträge, **1**; Jaeckle, Zeitschr. f. physiol. Chem., **36** (literature); Henriques and Hansen, Skand. Arch. f. Physiol., **11**; Leick and Winkler, Arch. f. Path. u. Pharm., **48**.

[3] Zeitschr. f. physiol. Chem., **65**.

found the administered fat in the tissues. HOFMANN starved dogs until they appeared to have lost their fat, and then fed them upon large quantities of fat and only little proteins. When the animals were killed he found so large a quantity of fat that it could not have been formed from the administered proteins alone, but the greater part must have been derived from the fat of the food. PETTENKOFER and VOIT arrived at similar results in regard to the action of the absorbed fats in the organism, though their experiments were of another kind. MUNK found that on feeding with free fatty acids, these are deposited in the tissues, not, however, as such; but they are transformed by synthesis with glycerin into neutral fats on their passage from the intestine into the thoracic duct. The connection between the fat of the food, and of the body has also been shown by others, especially ROSENFELD. CORONEDI and MARCHETTI and in particular WINTERNITZ [1] have shown that iodized fat is taken up in the intestinal tract and deposited in the various organs.

Proteins and carbohydrates are considered as the mother-substances of the fats formed in the organism.

The formation of the so-called *corpse-wax, adipocere*, which consists of a mixture of fatty acids, ammonia, and lime-soaps, from parts of the corpse rich in proteins, is sometimes given as a proof of the *formation of fats from proteins*. The accuracy of this view has, however, been disputed, and many other explanations of the formation of this substance have been offered. According to the experiments of KRATTER and K. B. LEHMANN, it seems as if it were possible by experimental means to convert animal tissue rich in proteins (muscles) into adipocere by the continuous action of water. Irrespective of this, SALKOWSKI has shown that in the formation of adipocere, the fat itself takes part, in that the olein decomposes with the formation of solid fatty acids, still it must be considered that lower organisms undoubtedly take part in its formation. The production of adipocere as a proof of the formation of fat from proteins is disputed by many investigators for this and other reasons.

Fatty degeneration has been considered as another proof of the formation of fat from proteins. From the investigations of BAUER on dogs, and LEO on frogs, it was assumed that, at least in acute poisoning by phosphorus, a fatty degeneration, with the formation of fat from proteins, takes place. PFLÜGER has raised such strong arguments against the older researches as well as the more recent one of POLIMANTI, who claims to have shown the formation of fat from proteins in phosphorus

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[1] Coronedi and Marchetti, cited by Winternitz, Zeitschr. f. physiol. Chem., 24. A review of the literature on fat formation may be found in Rosenfeld, Fettbildung. in Ergebnisse der Physiologie, 1, Abt. 1.

poisoning, that we cannot consider the formation of fat as conclusively proved. The investigations of LEBEDEFF, ATHANASIU, TAYLOR, SCHWALBE and others, have shown that probably no new formation of fat from protein took place, but rather a fat migration and that this is actually the case has been especially shown by ROSENFELD and recently by SHIBATA [1] in a conclusive manner.

Another more direct proof of the formation of fat from proteins has been given by HOFMANN. He experimented with fly-maggots. A number of these were killed and the quantity of fat determined. The remainder were allowed to develop in blood whose proportion of fat had been previously determined, and after a certain time they were killed and analyzed. He found in them from seven to eleven times as much fat as was contained in the maggots first analyzed and the blood taken together. PFLÜGER [2] has made the objection that a considerable number of lower fungi develop in the blood under these conditions, in whose cell-body fats and carbohydrates are formed from the different constituents of the blood and their decomposition products, and that these serve as food for the maggots.

WEINLAND [3] has observed the formation of higher non-volatile fatty acids in the Calliphora larvæ when they were rubbed to a homogeneous paste after the addition of Witte's peptone. This experiment shows a formation of fat from protein, but cannot be considered as quite conclusive.

As a more convincing proof of fat formation from proteins, the investigations of PETTENKOFER and VOIT are often quoted. These investigators fed dogs with large quantities of meat containing the least possible proportion of fat, and found all of the nitrogen in the excreta, but only a part of the carbon. As an explanation of these conditions it has been assumed that the protein of the organisms splits into a nitrogenized and a non-nitrogenized part, the former changing into the nitrogenized final product, urea, and like products, and the other part, on the contrary, being retained in the organism as fat (PETTENKOFER and VOIT).

PFLÜGER has arrived at the following conclusion by an exhaustive criticism of PETTENKOFER and VOIT'S experiments and a careful recalculation of their balance-sheet; that these very meritorious investiga-

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[1] Bauer, Zeitschr. f. Biologie, 7; Leo, Zeitschr. f. physiol. Chem., 9; Polimanti, Pflüger's Arch., 70; Pflüger, *ibid.*, 51 (literature on the formation of fat from protein) and 71; Athanasiu, *ibid.*, 74; Taylor, Journ. Exp. Medicine, 4; see also footnote 2, p. 384; Shibata, Bioch. Zeitschr., 37, which contains the literature; Rosenfeld, Ergebn. d. Physiol., 1.

[2] See Rosenfeld, Fettbildung, Ergebnisse der Physiologie, 1, Abt. 1.

[3] Zeitschr. f. Biol., 51 and 52.

tions, which were continued for a series of years, were subject to such great defects that they are not conclusive as to the formation of fat from proteins. He especially emphasizes the fact that these investigators started from a wrong assumption as to the elementary composition of the meat, and that the quantity of nitrogen assumed by them was too low and the quantity of carbon too high. The relation of nitrogen to carbon in meat poor in fat was assumed by VOIT to be as 1:3.68, while according to PFLÜGER it is 1:3.22 for fat-free meat after deducting the glycogen, and according to RUBNER 1:3.28 without deducting the glycogen. On recalculation of the figures, using these coefficients, PFLÜGER has arrived at the conclusion that the assumption as to the formation of fat from proteins finds no support in these experiments.

In opposition to these objections, E. VOIT and M. CREMER have made new feeding experiments, to show the formation of fat from proteins, but the proof of these recent investigations has been disputed by PFLÜGER. On feeding a dog on meat poor in fat (containing a known quantity of ether extractives, glycogen, nitrogen, water, and ash), KUMAGAWA[1] could not prove the formation of fat from protein. According to him the animal body under normal conditions has not the power of forming fat from protein.

Several French investigators, especially CHAUVEAU, GAUTIER, and KAUFMANN,[2] consider the formation of fat from proteins as positively proved. KAUFMANN has recently substantiated this view by a method which will be spoken of in detail in Chapter XVII, in which he studied the nitrogen elimination and the respiratory gas exchange in conjunction with the simultaneous formation of heat.

As we are agreed that carbohydrates and glycogen, as well as sugar, can be formed from proteins, the fact cannot be denied that possibly an indirect formation of fat from proteins, with a carbohydrate as an intermediate step, can take place. The possibility of a direct fat formation from proteins without the carbohydrate as intermediary must also be generally admitted, although such a formation has not been conclusively proved.

According to CHAUVEAU and KAUFMANN, in the direct formation of fat from proteins, the fat is formed besides urea, carbon dioxide, and water, as an intermediary product in the oxidation of the proteins, while GAUTIER considers the formation of fat from proteins as a cleavage without the taking up of oxygen. If fat is formed from protein in the animal body, then such formation is not a splitting off of fat from the

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[1] See Rosenfeld, Fettbildung, Ergebnisse der Physiologie, 1, Abt. 1.

[2] Kaufmann, Arch. de physiol., (5) 8, where the works of Chauveau and Gautier are cited.

proteins, but rather a synthesis from primarily formed cleavage products of proteins which are poor in carbon.

The *formation of fat from carbohydrates* in the animal body was first suggested by LIEBIG. This was opposed for some time, and until lately it was the general opinion that a direct formation of fat from carbohydrates not only had not been proved, but also that it was improbable. The undoubtedly great influence of the carbohydrates on the formation of fat as observed and proved by LIEBIG was explained by the statement, that the carbohydrates were consumed instead of the absorbed fat or that derived from the proteins, hence they have a sparing action on the fat. By means of a series of nutrition experiments [1] with different animals, with foods especially rich in carbohydrates it has been apparently proved that a direct formation of fat from carbohydrates does actually occur. The processes by which this formation takes place are still unknown. As the carbohydrates do not contain such complicated carbon chains as the fats, the formation of fat from carbohydrates must consist of a synthesis, in which the group CHOH is converted into CH_2; hence a reduction must occur.

After feeding with very large quantities of carbohydrates the relation between the inspired oxygen and the expired carbon dioxide, i.e., the respiratory quotient $\frac{CO_2}{O}$, was found greater than 1 in certain cases (HANRIOT and RICHET, BLEIBTREU, KAUFMANN, LAULANIÉ [2]). This is explained by the assumption that the fat is formed from the carbohydrate by a cleavage setting free carbon dioxide and water without taking up oxygen. This increase in the respiratory quotient also depends in part on the increased combustion of the carbohydrate.

When food contains an excess of fat, the superfluous amount is stored up in the fatty tissue, and on partaking of food deficient in fat this accumulation is quickly exhausted; and it is very probable that the lipase is of importance here, as LOEVENHART [3] has found that all over the body where fat is deposited in large amounts lipase also occurs in considerable amounts. There is perhaps not one of the various tissues that decreases so much in starvation as the fatty tissue. The organism, then, possesses in this tissue a depot where there is stored, during proper

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[1] Lawes and Gilbert, Phil. Transactions, 1859, part 2; Soxhlet, see Maly's Jahresber., **11**, 51; Tscherwinsky, Landwirthsch. Versuchsstaat, **29** (cited from Maly's Jahresber., **18**); Meissl and Stromer, Wien. Sitzungsber., **88**, Abt. 3; Schultze, Maly's Jahresber., **11**, 47; Chaniewski, Zeitschr. f. Biologie, **20**; Voit and Lehmann, see C. v. Voit, Sitzungsber, d. k. bayer. Akad. d. Wissensch., 1885; I. Munk, Virchow's Arch., **101**; Rubner, Zeitschr. f. Biologie, **22**; Lummert, Pflüger's Arch., **71**.

[2] Hanriot and Richet, Annal. de Chim. et de Phys. (6), **22**; Bleibtreu, Pflüger's Arch., **56** and **85**; Kaufmann, Arch. de Physiol. (5), **8**; Laulanié, *ibid.*, 791.

[3] Amer. Journ. of Physiol., **6**.

alimentation, a nutritive substance of great importance in the development of heat and vital force, which substance, on insufficient nutrition, is given up as may be needed. On account of their low conducting power, the fatty tissues become of great importance in regulating the loss of heat from the body. They also serve to fill cavities and act as a protection and support to certain internal organs.

CHAPTER X.

MUSCLES.

STRIATED MUSCLES.

In the study of the muscles the chief problem for physiological chemistry is to isolate their different morphological elements and to investigate each element separately. By reason of the complicated structure of the muscles this has been thus far almost impossible, and we must be satisfied at the present time with a few microchemical reactions in the investigation of the chemical composition of the muscular fibers.

Each muscle-tube or each muscle-fiber consists of a sheath, the SARCOLEMMA, which seems to be composed of a substance similar to elastin, and containing a large proportion of *protein*. This last, which in life possesses the power of contractility, has in the inactive muscle an alkaline reaction, or, more correctly speaking, an amphoteric reaction with a predominating action on red litmus paper. RÖHMANN found that the fresh, inactive muscle shows an alkaline reaction with red lacmoid, and an acid reaction with brown turmeric. From the effect of various acids and salts on these coloring-matters, he concludes that the alkalinity of the fresh muscle with lacmoid is due to sodium bicarbonate, diphosphate, and probably also to an alkaline combination of protein bodies, and the acid reaction with turmeric, on the contrary, to chiefly monophosphate. The dead muscle has an acid reaction, or, more correctly, the acidity with turmeric increases on the decease of the muscle, and the alkalinity with lacmoid decreases. The difference depends on the presence of a larger quantity of monophosphate in the dead muscle, and according to RÖHMANN free lactic acid is found in neither the one case nor the other.[1]

If the somewhat disputed statements relative to the finer structure of the muscles are disregarded, one can differentiate in the striated muscles between the two chief components, the doubly refracting—*anisotropous*—and the singly refracting—*isotropous*—substance. Both contain abundance of protein, which form the chief part of the solids of the muscles.

[1] The various reports in regard to the reaction of the muscles and the cause thereof are conflicting. See Röhmann, Pflüger's Arch., **50** and **55**; Heffter, Arch. f. exp. Path. u. Pharm., **31** and **38**. These references contain the pertinent literature.

If the muscular fibers are treated with reagents which dissolve proteins, such as dilute hydrochloric acid, soda solution, or gastric juice, they swell greatly and break up into "BOWMAN'S *disks*." By the action of alcohol, chromic acid, boiling water, or in general such reagents as cause a shrinking, the fibers split longitudinally into fibrils; and this behavior shows that several chemically different substances of various solubilities enter into the construction of the muscular fibers.

The protein myosin is generally considered as the principal constituent of the diagonal disks, while the isotropous substance contains the chief mass of the other proteins of the muscles as well as the chief portion of the extractives. According to the observations of DANILEWSKY, confirmed by J. HOLMGREN,[1] myosin may be completely extracted from the muscle without changing its structure, by means of a 5-per cent solution of ammonium chloride, which fact conflicts with the above view. DANILEWSKY claims that another protein-like substance, insoluble in ammonium chloride and only swelling up therein, enters essentially into the structure of the muscles. The proteins, which form the principal part of the solids of the muscles, are of the greatest importance.

Proteins of the Muscles.

Like the blood which contains a fluid, the blood-plasma, which spontaneously coagulates, separating fibrin and yielding blood-serum, so also the living muscle, at least of cold-blooded animals, contains, as first shown by KÜHNE, a spontaneously coagulating liquid, the muscle-plasma, which coagulates quickly, separating a protein body, myosin, and yielding also a serum. That liquid which is obtained by pressing the living muscle is called *muscle-plasma*, while that obtained from the dead muscle is called *muscle-serum*. These two fluids contain at least in part different protein bodies.

Muscle-plasma was first prepared by KÜHNE from frog-muscles, and later by HALLIBURTON, according to the same method, from the muscles of warm-blooded animals, especially rabbits. The principle of this method is as follows: The blood is removed from the muscles immediately after the death of the animal by passing through them a strongly cooled common-salt solution of 5–6 p. m. Then the muscles are quickly cut and immediately frozen thoroughly so that they can be ground in this state to a fine mass—"muscle-snow." This pulp is strongly pressed in the cold, and the liquid which exudes is called muscle-plasma. According to V. FÜRTH [2] this cooling or freezing is not necessary. It is sufficient to extract the muscle free from blood, as above directed, with a 6 p. m. common salt solution.

Muscle-plasma forms a yellow to brownish-colored fluid with an alkaline reaction. It varies in different animals. Muscle-plasma from the frog spontaneously coagulates, slowly, at a little above 0° C., but more

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[1] Danilewsky, Zeitschr. f. physiol. Chem., 7; J. Holmgren, Maly's Jahresber., 23.

[2] See Kühne, Untersuchungen über das Protoplasma, (Leipzig, 1864), 2; Halliburton, Journ. of Physiol., 8; v. Fürth, Arch. f. exp. Path. u, Pharm., 36 and 37; Hofmeister's Beiträge, 3, and Ergebnisse der Physiologie, 1, Abt. 1; Stewart and Sollmann, Journ. of Physiol., 24.

quickly at the temperature of the body. Muscle-plasma from mammals coagulates slowly, according to v. FÜRTH, even at the temperature of the room, though only slightly, and it can hardly be considered as a process comparable with the coagulation of the blood. Indeed the question may be asked whether a true muscle-plasma does exist in warm-blooded animals, or whether the fluid obtained from such muscles exactly represents the plasma of the living muscle. According to KÜHNE and v. FÜRTH the reaction remains alkaline during coagulation, while HALLIBURTON, STEWART and SOLLMANN find that it becomes acid. Earlier investigators held that the clot consists of a globulin called myosin, while v. FÜRTH claims that it consists of two coagulated proteins, myosin-fibrin and myogen-fibrin.

The study of the proteins of the muscles, as well as their nomenclature, has changed markedly in the last few years, and it is questionable whether an essential difference exists between the proteins of the muscle-plasma and the muscle-serum of warm-blooded animals. Nevertheless it is necessary to discuss separately the proteins of the dead muscle as well as those of the muscle-plasma.

The *proteins of the dead muscle* are in part soluble in water or dilute salt solutions, and in part are insoluble therein. Myosin and musculin and also myoglobulin and myoalbumin, which exist to a very slight extent and are perhaps only derived from the remaining lymph, belong to the first group, and the stroma substances of the muscle-tubes belong to the second group.

Myosin was first discovered by KÜHNE, and constitutes the principal mass of the soluble proteins of the dead muscle. It is generally considered as the most essential coagulation product of muscle-plasma. The name myosin, KÜHNE also gives to the mother-substance of the plasma-clot, and this mother-substance forms, according to certain investigators, the principal mass of contractile protoplasm. The findings as to the occurrence of myosin in other organs besides the muscles require further confirmation. The quantity of myosin in the muscles of different animals varies, according to DANILEWSKY,[1] between 30 and 110 p. m.

Myosin, as obtained from dead muscles, is a globulin whose elementary composition, according to CHITTENDEN and CUMMINS,[2] is, on an average, the following: C 52.28, H 7.11, N 16.77, S 1.27, O 22.03 per cent. If the myosin separates as fibers, or if a myosin solution with a minimum quantity of alkali is allowed to evaporate to a gelatinous mass on a microscope-slide, doubly refracting myosin may be obtained. Myosin has the general properties of the globulins and is readily converted into

[1] Zeitschr. f. physiol. Chem., 7.

[2] Studies from the Physiol. Chem. Laboratory of Yale College, New Haven, 3, 115.

albuminates by dilute acids or alkalies. It is completely precipitated upon saturation with NaCl, also by $MgSO_4$, in a solution containing 94 per cent of the salt with its water of crystallization (HALLIBURTON). The precipitated myosin readily becomes insoluble. Like fibrinogen it coagulates at 56° C. in a solution containing common salt, but differs from it, since under no circumstances can it be converted into fibrin. The coagulation temperature, according to CHITTENDEN and CUMMINS, not only varies for myosins of different origin, but also for the same myosin in different salt solutions.

Myosin may be prepared in the following way, as suggested by HALLIBURTON: The muscle is first extracted by a 5-per cent magensium-sulphate solution, and by fractional precipitation with magnesium sulphate the musculin and then the myosin are precipitated (see HALLIBURTON, l. c.).

The older and perhaps the usual method of preparation consists, according to DANILEWSKY,[1] in extracting the muscle with a 5–10 per cent ammonium-chloride solution, precipitating the myosin from the filtrate by strongly diluting with water, and redissolving the precipitate in ammonium-chloride solution, and the myosin obtained from this solution is reprecipitated either by diluting with water or by removing the salt by dialysis.

Musculin,[2] called PARAMYOSINOGEN by HALLIBURTON, and MYOSIN by v. FÜRTH, is a globulin which is characterized by its low coagulation temperature, in frogs below 40°, in mammalia 42–48°, and in birds about 51° C., and which may vary in different species of animals. It is more easily precipitated than myosin by NaCl or $MgSO_4$ (50 per cent salt, including water of crystallization). According to v. FÜRTH it is precipitated by ammonium sulphate with a concentration of 12–24 per cent salt. If the dead muscle is extracted with water a part of the musculin goes into solution, and may be precipitated therefrom by carefully acidifying. It separates from a dilute salt solution on dialysis. Musculin readily passes into an insoluble modification which v. FÜRTH calls *myosin fibrin*. Musculin is called myosin by v. FÜRTH, as he considers it nothing but myosin. As musculin has a lower coagulation temperature and has other precipitating properties for neutral salts than the older substance called myosin, it is difficult to accept this view.

Myoglobulin. After the separation of the musculin and the myosin from the salt extract of the muscle by means of $MgSO_4$, the myoglobulin may be precipitated

[1] Zeitschr. f. physiol. Chem., 5, 158.

[2] As we have up to the present no conclusive basis for the identity of the globulins called myosin and paramyosinogen, and also as the use of the name myosin for the last-mentioned substance may readily cause confusion, the author does not feel justified in dropping the old name musculin (Nasse).

by saturating the filtrate with the salt. It is similar to serglobulin, but coagulates at 63° C. (HALLIBURTON). *Myoalbumin*, or muscle-albumin, seems to be identical with seralbumin (seralbumin α, according to HALLIBURTON), and probably originates only from the blood or the lymph. Proteoses and peptones do not seem to exist in the fresh muscles.

After the complete removal from the muscle of all protein bodies which are soluble in water and ammonium chloride, an insoluble protein remains which only swells in ammonium-chloride solution, and which forms with the other insoluble constituents of the muscular fiber the "*muscle-stroma.*" According to DANILEWSKY the amount of such stroma substance is connected with the muscle activity. He maintains that the muscles contain a greater amount of this substance, compared with the myosin present, when the muscles are quickly contracted and relaxed, the correctness of which report has recently been disputed by SAXL.[1]

According to J. HOLMGREN,[2] this stroma substance does not belong to either the nucleoalbumin or the nucleoprotein group. It is not a glucoproteid, as it does not yield a reducing substance when boiled with dilute mineral acids. It is very similar to the coagulable proteins, and dissolves in dilute alkalies, forming an albuminate. The elementary composition of this substance is almost the same as that of myosin. There is no doubt that the insoluble substances, myofibrin and myosin fibrin, which are formed, according to V. FÜRTH, in the coagulation of the plasma, also occur among the stroma substances. When the muscles are previously extracted with water, the stroma substances also contain a part of the myosin hereby made insoluble. The observations of SAXL on rabbits' muscles agree with this view that the fresh muscle after work contains 11.5–21.6 per cent of the total protein in an insoluble form, while the muscle after rigor mortis contains on the contrary 71.5–73.2 per cent.

To the proteins insoluble in water, and neutral salts, belongs the *nucleoprotein* detected by PEKELHARING, which occurs as traces and is soluble in faintly alkaline water, and which probably originates from the muscle nuclei. According to BOTTAZZI and DUCCESCHI[3] the heart muscle is richer in nucleoprotein than the skeletal muscle.

Muscle-syntonin, which may be obtained by extracting the muscles with hydrochloric acid of 1 p.m., and which, according to K. MÖRNER, is less soluble and has a greater aptitude to precipitate than other acid albumins, seems not to occur preformed in the muscles. HEUBNER'S[4] *mytolin* is modified muscle-proteid, chiefly myosin, which has lost a part of its sulphur by the action of alkali.

Proteins of the Muscle-plasma. As above stated, myosin was ordinarily considered as the coagulated modification of a soluble protein existing in the muscle-plasma. As in blood-plasma there is present a mother-substance of fibrin, fibrinogen, so also there exists in the muscle-plasma a mother-substance of myosin, a soluble myosin or a *myosinogen*. This body has not thus far been isolated with certainty.

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[1] Hofmeister's Breitäge, 9.

[2] See footnote 1, p. 566.

[3] Pekelharing, Zeitschr. f. physiol. Chem., 22; Bottazzi and Ducceschi, Centralbl. f. Physiol., 12.

[4] Arch. f. exp. Pathol. u. Pharm., 58.

HALLIBURTON, who has detected in the muscles an enzyme-like substance, "*myosin ferment*," which is related to fibrin ferment but is not identical with it, has also found that a solution of purified myosin, in dilute salt solution (5 per cent $MgSO_4$), and sufficiently diluted with water, coagulates after a certain time, and at the same time becomes acid, and a typical myosin-clot separates. This coagulation, which is accelerated by warming or by the addition of myosin ferment, is, according to HALLIBURTON, a process analogous to the coagulation of the muscle-plasma. According to this same investigator, myosin when dissolved in water by the aid of a neutral salt is reconverted into myosinogen, while after diluting with water myosin is again produced from the myosinogen. The musculin (paramyosinogen) is carried down, according to HALLIBURTON, with the myosin-clot, but has nothing to do with the coagulation, as the myosin-clot also forms in the absence of musculin, and this last is not changed into myosin.

Besides the traces of globulin and albumin, which perhaps do not belong to the muscle-plasma, there occur in mammals, according to v. FÜRTH, two proteins, namely, musculin (myosin according to v. FÜRTH) and myogen.

MUSCULIN (NASSE) = paramyosinogen (HALLIBURTON) = myosin (v. FÜRTH) forms about 20 per cent of the total proteins of the muscle-plasma of rabbits. Its properties have already been given, and it is sufficient to remark that its solutions become cloudy on standing, and a precipitate of *myosin fibrin* occurs, which is insoluble in salt solutions.

Myogen, or MYOSINOGEN (HALLIBURTON), forms the chief mass, 75–80 per cent, of the proteins of rabbit muscle-plasma. It does not separate from its solutions on dialysis and is not a true globulin, but a protein *sui generis*. It coagulates at 55–65° C. and is precipitated in the presence of 26–40 per cent ammonium sulphate. Myogen solutions are precipitated by acetic acid only in the presence of some salt. It is converted into an albuminate by alkalies, this albuminate being precipitable by ammonium chloride. Myogen passes spontaneously, especially with higher temperatures as well as in the presence of salt, into an insoluble modification, *myogen fibrin*. A protein, coagulating at 30–40° C., *soluble myogen fibrin*, is produced as a soluble intermediate step. This substance occurs to a considerable extent in native frog-muscle plasma. It does not always occur in the muscle-plasma of warm-blooded animals, and when it does it is present only to a slight extent. It can be separated by precipitating with salt or by diffusion. HALLIBURTON'S assumption as to the action of a special myosin ferment has not sufficient basis, according to v. FÜRTH, nor has the often-admitted analogy with the coagulation of the blood. The difference between the musculin and the myogen in their becoming insoluble is that the musculin passes into myosin fibrin without any soluble intermediate steps.

Myogen may be prepared, according to v. FÜRTH, by heating, for a short time, the dialyzed and filtered plasma to 52° C., separating it in

this way from the rest of the musculin. The myogen exists in the new filtrate and can be precipitated by ammonium sulphate. The musculin may also be removed by adding 28 per cent ammonium sulphate and then precipitating the myogen from the filtrate by saturating with the salt.

STEWART and SOLLMANN admit of only two soluble proteins in the muscles. One is the paramyosinogen, which is the same as v. FÜRTH's myosin+the soluble myogen fibrin. The other they call myosinogen, which corresponds to v. FÜRTH's myogen or to HALLIBURTON's myosinogen+myoglobulin. It is a typical globulin which coagulates at 50–60° C. The paramyosinogen as well as the myosinogen is readily converted into an insoluble modification, myosin. The myosin of the above investigators is the same as v. FÜRTH's myosin fibrin+myogen fibrin, and corresponds, it seems, also to myosin mixed with paramyosinogen (HALLIBURTON). STEWART and SOLLMANN differ from HALLIBURTON in considering that paramyosinogen also coagulates and is converted into myosin. According to them myosin is also insoluble in a NaCl solution.

The views of the various investigators differ so essentially and the nomenclature is so complicated (three different things are designated by the name myosin) that it is extremely difficult to give any correct review of the various opinions.[1] Thorough investigations on this subject are very necessary.

Myoproteid is a protein found by v. FÜRTH in the plasma from fish-muscles. It does not coagulate on boiling, is precipitated by acetic acid, and is considered as a compound protein by v. FÜRTH.

In connection with v. FÜRTH's work, PRZIBRAM has carried on ivestigations on the occurrence of muscle-proteins in various classes of animals. The myosin (v. FÜRTH) and myogen occur in all classes of vertebrates; the myogen is always absent in the invertebrates. Myoproteid occurs, at least in considerable quantity, only in fishes. In the muscle after cutting the nerve, STEYRER [2] found somewhat more musculin and less myogen in the muscle-juice than in the normal muscle.

Muscle-pigments. There is no question that the red color of the muscles, even when completely freed from blood, depends in part on hæmoglobin. K. MÖRNER has shown that muscle-hæmoglobin is not quite identical with blood-hæmoglobin. The statement of MACMUNN that in the muscles another pigment occurs which is allied to hæmochromogen, and called *myohæmatin* by him, has not been substantiated, at least for muscles of higher animals (LEVY and MÖRNER [3]). MACMUNN claims that myohæmatin occurs in the muscles of insects, which do not contain any hæmoglobin. The reddish-yellow coloring-matter of the muscles of the salmon has been little studied.

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[1] For these reasons the author is not sure whether he has understood and correctly given the work of the different investigators.

[2] Przibram, Hofmeister's Beiträge, **2**; Steyrer, *ibid.*, **4**.

[3] See MacMunn, Phil. Trans. of Roy. Soc., **177**, part 1, Journ. of Physiol., 8 and Zeitschr. f. Physiol. Chem., **13**; Levy, *ibid.*, **13**; K. Mörner, Nord. Med. Archiv. Festband., 1897, and Maly's Jahresber., **27**.

Various enzymes have been found in the muscles. To these belong (besides traces of fibrin ferment and myosin ferment?) the *catalases* and *oxidases*, which occur only to a slight extent and the *glycolytic enzyme* (Chapter VII). An *amylolytic* and a *proteolytic enzyme* (HEDIN and ROWLAND [1]) have also been found, and the hydrolytic and oxidizing enzymes (Chapter XIV) active in the formation and destruction of uric acid are also present.

Extractive Bodies of the Muscles.

The *nitrogenous extractives* in the muscles of higher animals consist chiefly of *creatine* and *creatinine* (especially in fishes) and *carnosine*. To these also belong *inosinic acid* (and the closely related *carnine*), *phosphocarnic acid*, *carnitine* and *purine bases*, especially *hypoxanthine*. The purine bases occur partly free (which is especially the case with hypoxanthine) and partly combined.

Among the extractive substances is also found the acid noticed by LIMPRICHT in the flesh of certain cyprinidea, namely, the nitrogenized *protic acid*, while the *isocreatinine* found by J. THESEN in fish-flesh is nothing but impure creatinine, according to POULSSON, SCHMIDT and KORNDÖRFER.[2] The following have also been found in the muscles, in certain cases only, of a few varieties of animals: *uric acid* (especially in alligators), *taurine* (in cephalopoda and oysters), *glycocoll* (in gasteropoda), *betaine* and *methyl guanidine*, in fish meat, several *monamino acids* and also the three hexone bases *histidine*, *lysine* and *arginine*.[3] *Urea* occurs in large quantities in the muscle of the shark and ray. The reports are very contradictory in regard to the occurrence of urea in the muscles of higher animals. According to the investigations of KAUFMANN and SCHÖNDORFF, confirmed by BRUNTON-BLAIKIE,[4] urea is a regular constituent of the muscles, although M. NENCKI and KOWARSKI dispute this.

In regard to the division of the nitrogenous extractives of the muscles, v. FÜRTH and SCHWARZ found the following in 1000 grams of the moist extremity musculature of the horse and dog (after subtracting the proteoses derived by secondary cleavage processes), 3.27–3.82 gram extractive nitrogen. Of this 4.5–7 per cent was ammonia, 6.1–11.1 per cent purine bodies, 26.5–37.1 per cent creatine and creatinine, 30.3–36.3 per cent carnosine fraction, 8.2–15.3 per cent base residue (carnitine, methylguanidine, etc.) and 6.3–16 per cent urea, polypeptides and amino-acids. The quantity of purine base nitrogen, according to BURIAN and HALL in fresh meat of the horse, ox and calf, was 0.55 p. m., 0.63

[1] Zeitschr. f. physiol. Chem., **32**.

[2] See Limpricht, Annal. d. Chem. u. Pharm., **127**, and Thesen, Zeitschr. f. physiol. Chem., **24**; Poulsson, Arch. f. exp. Path. u. Pharm., **51**; Schmidt and Korndörfer, *ibid.*, **51**.

[3] In regard to the extractives of the muscles see besides the specially cited works, Kurkenberg and Wagner, Zeitschr. f. biol. **21**; U. Suzuki and collaborators, Zeitschr. f. physiol. Chem. **62** and Chem. Centralbl. 1913, **1**; Suwa, Pflüger's Arch. **128** and **129**; Zunz, Centralbl. f. Physiol., **18**.

[4] Kaufmann, Arch. de Physiol. (5), **6**; Schöndorff, Pflüger's Arch., **62**; Nencki and Kowarski., Arch. f. exp. Path. u. Pharm., **36**; Brunton-Blaikie, Journ. of Physiol., **23**, Supplement.

p. m. and 0.71 p. m. respectively, which corresponds closely to the results found by SCAFFIDI, BUGLIA and COSTANTINO for the striated muscle of the calf, namely, 0.58–0.68 p. m. According to RINALDI and SCAFFIDI [1] the lowest values for the purine nitrogen occur in the striated muscles of the covering of polype, 0.436 p. m., then in fishes 0.595–0.82 p. m. and the highest 1.061 p. m. in birds. BUGLIA and COSTANTINO have determined the nitrogen titratable with formol, and from this determined the amount of monamino-acid nitrogen as well as diamino-acid nitrogen in various animals. In oxen they found in the moist, striated muscle 0.18 p. m. monamino- and 0.40 p. m. diamino-nitrogen. In the heart the corresponding figures were 0.18 and 0.18 p. m. In percentage of the total nitrogen the total amino-acid nitrogen in the striated muscle was 1.70 per cent and in the heart 1.48 per cent.

The most extensively occurring nitrogenous extractives in the muscle are creatine and carnosine.

Creatine, $C_4H_9N_3O_2$, $C\begin{cases} NH_2 \\ =NH \\ N(CH_3).CH_2COOH \end{cases}$, or methyl-guanidine-acetic acid, occurs in the striated as well as smooth muscles. In the striated muscle of vertebrates the amount varies between 2.5 and 7 p. m. It is also found in the brain, blood, transudates, amniotic fluid, and sometimes also in the urine.

Creatine may be prepared synthetically from cyanamide and sarcosine (methylglycocoll). On boiling with baryta-water it decomposes, with the addition of water, and yields urea, sarcosine, and certain other products. Because of this behavior several investigators consider creatine as a step in the formation of urea in the organism. On boiling with acids, creatine is easily converted, with the elimination of water into the corresponding anhydride, creatinine, $C_4H_7N_3O$, which is retransformed into creatine by the action of alkali.

The question as to the mutual relation of creatine to creatinine in metabolism will be treated in Chapter XIV (urine). In this chapter, besides the properties and reactions, we will discuss the question as to the origin of creatine and its relation to the metabolism of the muscles.

Of special interest in this regard, besides the relation between creatine and muscle work which will be discussed below, is the question as to the occurrence of free or combined creatine in the muscle. URANO by the aid of dialysis experiments has shown the probability that the creatine does not exist free in the muscle, but as a labile, non-dialyzable combination. Nevertheless GOTTLIEB and STANGASSINGER claim by various researches to have shown in the autolysis of muscles and other organs, that creatine is first formed and then first changed into creatinine by special bodies of an enzymotic nature, and then destroyed. SEEMANN

[1] v. Fürth and Schwarz, Bioch. Zetschr., **30**; Scaffidi, *ibid.*, **33**; Burian and Hall, Zeitschr. f. physiol. Chem. **38**; Buglia and Costantino, *ibid.*, **81** and **82**; Rinaldi and Scaffidi, Bioch. Zeitschr., **41**.

claims, by an autolysis of three months' duration, to have obtained two to three times as much creatinine, directly from the muscle, and after the addition of creatinine-free-gelatin four times as much, which is an argument against the enzymotic destruction of creatinine in autolysis, and he admits of the formation of creatine (or creatinine) from protein. The autolytic experiments of ROTHMANN also indicate the formation of creatine from a preliminary body, and the recent experiments of VAN HOOGENHUYZE and VERPLOEGH make the enzymotic transformation of creatine and creatinine probable. MELLANBY positively denies the re-formation of creatine as well as its destruction in autolysis entirely free from bacteria. It is hard to draw positive conclusions from experiments with autolysis. The transfusion experiments of GOTTLIEB and STANGASSINGER with the kidneys and livers of dogs, not only point to the ability of these organs to decompose creatine, but also for a re-formation of creatine in the liver. Further investigations are still very necessary, especially as the conditions are probably not the same in all animals. Thus NOEL-PATON and MACKIE[1] found that the exclusion of the liver in birds is without influence upon the creatine metabolism.

As will be discussed in Chapter XIV, no certain relationship exists between the quantity of food protein and the extent of creatine and creatinine elimination. On the contrary, several observations speak for a relation between creatine formation and catabolism of organ protein, especially muscle protein and according to NOEL-PATON[2] the elimination of creatine in bird urine, which here corresponds to the creatinine in the mammalian urine, is a measure of the protein catabolism of the muscles.

Under all circumstances the proteins, and especially the guanidine groups contained therein, are the mother-substance for the creatine or creatinine. The guanidine occurs in the protein molecule as arginine; but according to OTORI it is not improbable that in the protein also other guanidine groups exist. Nevertheless the observations of JAFFÉ[3] speak against the assumption as to a creatine formation from arginine as he found that arginine subcutaneously injected did not cause any increase in the elimination of creatine substances. But as the introduced arginine was probably decomposed by the enzyme arginase, because the urea elimination was greatly increased,[4] does not exclude

[1] Urano, Hofmeister's Beiträge, **9**; Gottlieb and Stangassinger, Zeitschr. f. physiol. Chem., **52** and **55**; Stangassinger, *ibid.*, **55**; Seemann, Zeitschr. f. Biol., **49**; Rothmann, Zeitschr. f. physiol. Chem., **57**; v. Hoogenhuyze and Verploegh, *ibid.*, **57**; Mellanby, Journ. of Physiol., **36**; Noel-Paton and Mackie, Journ. of Physiol. **45**.

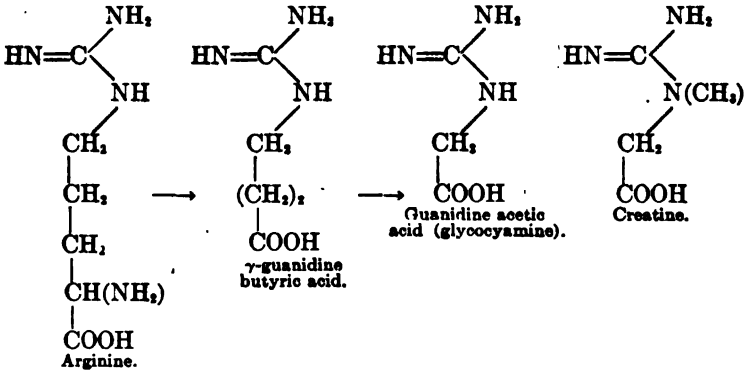
[2] Journ. of Physiol., 39.

[3] Otori, Zeitschr. f. physiol. Chem., **42**, **43**; Jaffé, *ibid.*, **48**.

[4] See Thompson, Journ. of Physiol., **32** and **33**.

the possibility that in the muscles, which according to KOSSEL and DAKIN contain only little arginase, the arginine was decomposed in other ways. In autolysis as well as perfusion experiments with livers, INOUYE [1] has recently shown that an increase in the creatine occurs at the expense of the arginine added.

Starting with the observation of Jaffé [2] that glycocyamine (guanidine acetic acid) in rabbits is transformed with a methylation into creatine, we can consider the cleavage of arginine into creatine in the following manner, basing this conception upon the ruling conception on the cleavage of amino-acids and fatty acids in the animal body.



The opinions are not unanimous in regard to the organ producing creatine or creatinine. Based upon several investigations it is generally admitted that the liver here plays an important rôle. Several other organs may also be considered and in the first place, the muscles. According to MELLANBY the creatinine is probably formed in the liver, transformed into creatine in the muscles and there stored up as such. Other observations still speak for the fact that the creatine is formed in the muscles and transformed into creatinine in the liver, while according to NOEL-PATON and MACKIE the exclusion of the liver in birds is without effect upon the creatinine metabolism.

Creatine crystallizes in hard, colorless, monoclinic prisms which lose their water of crystallization at 100° C. It is soluble in 74 parts of water at the ordinary temperature, and in 9419 parts absolute alcohol. It dissolves more easily with the aid of heat. Its watery solution has a neufral reaction. Creatine is not dissolved by ether. If a creatine solution is boiled with precipitated mercuric oxide, this is reduced, especially in the presence of alkali, to mercury and oxalic acid, and the foul-smelling methyluramine (methylguanidine) is developed. A solu-

[1] Kossel and Dakin, Zeitschr. f. physiol. Chem., 41 and 42; Inouye, *ibid.*, 81.
[2] Zeitschr. f. physiol. Chem., 48; see also Dorner, *ibid.*, 52.

tion of creatine in water is not precipitated by basic lead acetate, but gives a white, flaky precipitate with mercurous nitrate if the acid reaction is neutralized. When boiled for an hour with dilute hydrochloric acid, creatine is converted into creatinine, and may be identified by its reactions. On boiling with formaldehyde it can be transformed into dioxymethylenecreatinine, which crystallizes readily (JAFFÉ [1]).

The preparation and detection of creatine is best accomplished by the following method of NEUBAUER,[2] which was first used in the preparation of creatine from muscles: Finely cut meat is extracted with an equal weight of water at 50–55° C. for 10–15 minutes, pressed, and extracted again with water. The proteins are removed from the united extracts so far as possible by coagulation at boiling heat, the filtrate precipitated by the careful addition of basic lead acetate, the lead removed from this filtrate by H_2S, and the solution then carefully concentrated to a small volume. The creatine, which crystallizes in a few days, is collected on a filter, washed with alcohol of 88 per cent, and purified, when necessary, by recrystallization. In the preparation of large quantities of creatine we can especially start with meat extracts. The quantitative estimation of creatine is performed by transforming it into creatinine (see Chapter XIV).

Carnosine, $C_9H_{14}N_4O_3$, is a base first isolated by GULEWITSCH and AMIRADŽIBI from meat extracts and which subsequently was also prepared directly from meat. The quantity seems to be relatively considerable, as according to the above-mentioned determination of v. FÜRTH and SCHWARZ, the carnosine fraction from the horse and dog muscles was just as large or indeed greater than the creatine-creatinine fraction of the extractive nitrogen. KRIMBERG found 1.3 p. m. and SKWORZOW[3] 1.76 p. m. (as nitrate) in fresh meat.

Carnosine, which according to GULEWITSCH is identical with the base *ignotine* isolated from meat extracts by KUTSCHER while both bases are isomeric bodies according to KUTSCHER,[4] is a histidine derivative according to GULEWITSCH which on cleavage yields β-alanine besides histidine.

Carnosine is a base readily soluble in water, which is precipitated as stellar warts of short delicate needles from the concentrated watery solution by the addition of alcohol. The specific rotation for the light $\lambda = 546$ is according to GULEWITSCH in watery solution where $c = 12.925$ per cent and 20.1° C. $= +25.3°$. The base is precipitated by phospho-

[1] Ber. d. d. Chem. Gesellsch., **35**.

[2] Zeitschr. f. analyt. Chem., **2** and **6**.

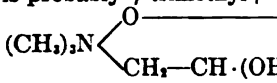
[3] Gulewitsch and Amiradžibi, Zeitschr. f. physiol. Chem., **30**; Gulewitsch, *ibid.*, **50, 51, 52** and **73**; Krimberg, *ibid.*, **48**; Skworzow, *ibid.*, **68**.

[4] Gulewitsch and Amiradžibi, Zeitschr. f. physiol. Chem., **30**; Gulewitsch, *ibid.*, **50, 51, 52** and **73**; Krimberg, *ibid.*, **68**; Skworzow, *ibid.*, **68**; Kutscher, *ibid.*, **50, 51.**

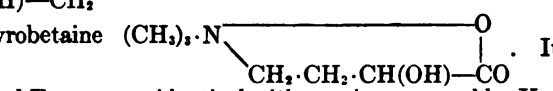
tungstic acid, by mercuric nitrate and by silver nitrate with an excess of barium hydrate. Carnosine-silver is soluble with difficulty in cold water but readily soluble in hot water. Carnosine nitrate melts at 211–212° C. Carnosine also gives a crystalline copper salt.

The principle in preparing this base consists in precipitating with phosphotungstic acid, separating the free base with barium hydrate, conversion into the nitrate, precipitating with silver nitrate and barium hydrate, decomposing the salt with H_2S and conversion into nitrate. From the latter, which is readily obtained as crystals, the base is precipitated by phosphotungstic acid and then set free by barium hydrate.

Carnitine, $C_7H_{15}NO_3$ (or $C_7H_{16}NO_3$), another base isolated by GULEWITSCH and KRIMBERG from meat extracts, has a strong alkaline reaction, is very readily soluble in water, and was also found by KRIMBERG in fresh meat. SKWORZOW found 0.19 p. m. carnitine in calf's muscles. Carnitine according to KRIMBERG is probably γ-trimethyl-β-oxybutyrobetaine with the formula



According to ENGELAND it is on the contrary a γ-trimethyl-α-oxybutyrobetaine



It is according to KRIMBERG and ENGELAND[1] identical with *novaine* prepared by KOSSEL from meat extracts. It gives crystalline double compounds with platinum, gold and mercuric chlorides, among which the following, $C_7H_{15}NO_2 2HgCl_2$, with a melting-point of 196–197° C., is especially used in the isolation of the base. The hydrochloride and the nitrate are readily soluble and the solution of the first is lævo-rotatory, about $(\alpha)_D = -21°$.

The inosinic acid has been discussed in Chapter II. In close relation to this stands probably the carnine.

Carnine, $C_7H_8N_4O_3 + H_2O$, is one of the substances found by WEIDEL in American meat extract. It has also been found by KRUKENBERG and WAGNER in frog muscles and in the flesh of fishes, and by POUCHET in the urine. Carnine is, according to HAISER and WENZEL,[2] probably only an equimolecular mixture of hypoxanthine and the crystalline pentoside (hypoxanthin-riboside) *inosine*, which is readily split by acid into hypoxanthine and pentose.

Carnine has been obtained as a white crystalline mass. It dissolves with difficulty in cold water, but more readily in warm. It is insoluble in alcohol and ether. It dissolves in warm hydrochloric acid and yields a salt crystallizing in shining needles, which gives a double compound with platinum chloride. Its watery solution is precipitated by silver nitrate, but this precipitate is dissolved neither by ammonia nor by warm nitric acid. Its watery solution is precipitated by basic lead acetate; but the lead compound may be dissolved on boiling.

Phosphocarnic acid[3] is a complicated substance, first isolated by SIEGFRIED

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[1] Gulewitsch and Krimberg, Zeitschr. f. physiol. Chem. **45**; Krimberg, *ibid.*; **49**, **50**, **53** and **56**, Ber. d. d. Chem. Gesellsch. **42**; Engeland, *ibid.*, **42**; Skworzow, l. c.

[2] Weidel, Annal. d. Chem. u. Pharm., **158**; Krunkenberg and Wagner, Sitzungsber. d. Würzb. phys.-med. Gesselsch., 1883; Pouchet, cited from Neubauer-Huppert, Analyse des Harnes, 10. Aufl., 335; Haiser and Wenzel, Monatsch. f. Chem., **29**.

[3] In regard to carnic acid and phosphocarnic acid, see the works of Siegfried, Arch. f. (Anat. u.) Physiol., 1894, Ber. d. deutsch. chem. Gesellsch., **28**, and Zeitschr. f. physiol. Chem., **21** and **28**; M. Müller, *ibid.*, **22**; Krüger, *ibid.*, **22** and **28**; Balke and

from meat extracts, which yields as cleavage products succinic acid, paralactic acid, carbon dioxide, phosphoric acid, and a carbohydrate group, besides the previously mentioned carnic acid, which is identical with or nearly related to antipeptone. It stands, according to SIEGFRIED, in close relation to the nucleins, and as it yields peptone (carnic acid), it is designated as a *nucleon* by SIEGFRIED. Phosphocarnic acid may be precipitated as an iron compound, *carniferrine*, from the extract of the muscles free from proteins. The quantity of phosphocarnic acid, calculated as carnic acid, can be determined by multiplying the quantity of nitrogen in the compound by the factor 6.1237 (BALKE and IDE). In this way SIEGFRIED found 0.57–2.4 p. m. carnic acid in the resting muscles of the dog, and M. MÜLLER 1–2 p. m. in the muscles of adults and a maximum of 0.57 p. m. in those of new-born infants. According to CAVAZZANI nucleon occurs to a much greater extent in oysters, namely, an average of 3.725 p. m. It also occurs, as he and MANICARDI found, in the plant kingdom. Phosphocarnic acid has not been prepared in the pure state and possesses on this account a variable composition; according to SIEGFRIED it serves as a source of energy in the muscles and is consumed during work. Besides, by means of its property of forming soluble salts with the alkaline earths, as also an iron combination soluble in alkalies, it acts as a means of transportation for these bodies in the animal body.

Phosphocarnic acid is prepared from the extract free from protein by first removing the phosphate by $CaCl_2$ and NH_3. The acid is precipitated as carniferrine by ferric chloride from the filtrate while boiling.

From LIEBIG'S extract of beef KUTSCHER has isolated besides the above-mentioned *ignotine* and *novaine*, several other bodies, *neosine*, $C_6H_{17}NO_2$, which according to KUTSCHER and ACKERMANN is a homologue of choline, *vitiatine* (as gold salt, $C_5H_{14}N_2.2HCl.2AuCl_3$), *carnomuscarine*, *methylguanidine* (also found by GULEWITSCH), *oblitine*, $C_{18}H_{28}N_2O_5$, which probably contains two novaine groups, which corresponds well with KRIMBERG'S view, and also *choline* and *neurine*. From dog muscles ACKERMANN[1] has isolated a platinum compound, $C_{11}H_{20}N_2O_4PtCl_6$, of a base called *myocynine*, which seems to be a hexamethylornithine. MICKO[2] found in meat extracts small quantities of alanine, glutamic acid, taurine and inosite, but no dipeptides. In crab extract KUTSCHER and ACKERMANN found no creatine and creatinine, but among others betaine and two new bases, *crangitine*, $C_{11}H_{20}N_2O_4$, and *crangonine*, $C_{13}H_{28}N_2O_2$. In crab muscles SUZUKI[3] and collaborators found a base, *canirine* which although it has the same composition, $C_6H_{14}N_2O_2$, as lysine, is not identical therewith.

The base *musculamine*, isolated by ETARD and VILA on the hydrolysis of veal, is nothing but cadaverine, according to POSTERNAK.[4]

We must also include among the nitrogenous extractives those bodies which were first discovered by GAUTIER,[5] and which occur only in very small quantities, namely, the leucomaines, *xanthocreatinine*, $C_5H_{10}H_4O$, *crusocreatinine*, $C_5H_8N_4O$, *amphicreatine*, $C_9H_{19}N_7O_4$, and *pseudoxanthine*, $C_4H_5N_5O$.

Ide, *ibid.*, **21**, and Balke, *ibid.*, **22**; Macleod, *ibid.*, **28**; E. Cavazzani, Centralbl. f. Physiol., **18**, 666; Panella, Maly's Jahresber., **34**.

[1] Kutscher, Zeitschr. f. Unters. d. Nahrungs- u. Genussmittel, **10**, **11**, Centralbl. f. Physiol., **19** and **21**, Zeitschr. f. physiol. Chem., **48**, **49**, **50**, **51**, with Ackermann, *ibid.*, **56**; Gulewitsch. *ibid.*, **47**; Krimberg, *ibid.*, **56**; Ackermann (on myocynine). Zeitschr. f. biol. **59**.

[2] Zeitschr. f. physiol. Chem., **56**.

[3] Kutscher and Ackermann, Zeitschr. f. Unters. d. Nahrungs- u. Genusmittel, **13** and **14**; Suzuki, Chem. Centralbl. 1913, **1**.

[4] Etard and Vila, Compt. Rend., **135**; Posternak, *ibid.*, **135**.

[5] See Maly's Jahresber., **16**, 523.

In the analysis of meat, and for the detection and separation of the various extractive bodies of meat, we make use of the systematic method as suggested by GAUTIER,[1] for details of which the reader is referred to the original article as well as for the Kutscher method for working the meat extracts.

The **non-nitrogenous extractive bodies** of the muscles are *inosite*, *glycogen*, *sugar*, and *lactic acid*.

Inosite, $C_6H_{12}O_6+H_2O=C_6H_6(OH)_6+H_2O$. This body, discovered by SCHERER, is not a carbohydrate, but belongs to the hydroaromatic compounds, and is a hexahydroxybenzene (MAQUENNE[2]). That it stands in certain relation to the carbohydrates follows from the fact that NEUBERG obtained some furfurol from inosite by distillation with phosphoric anhydride, and also that P. MEYER[3] found fermentation lactic acid in the urine of rabbits after the introduction of inosite *per os*. It has been known for some time that inosite undergoes lactic acid fermentation. The acid formed thereby is sarcolactic acid according to HILGER and fermentation lactic acid according to VOHL.[4]

Inosite is found in the muscles, liver, spleen, leucocytes, kidneys, suprarenal capsule, lungs, brain, testicles, and in the urine in pathological cases, and as traces in normal urine. It is found very widely distributed in the vegetable kingdom, especially in the unripe fruit of green beans (Phaseolus vulgaris), and therefore it is also called PHASEOMANNITE. In the plant kingdom another substance occurs which is called *phytin* and which is the Mg and Ca compound of inosite and phosphoric acid and which was first isolated by POSTERNAK. WINTERSTEIN identified this as an inosite-phosphoric acid. This inosite-phosphoric acid can be split into phosphoric acid and inosite by the plant enzyme *phytase* (SUZUKI, YOSHIMURA and TAKAISHI) as well as by enzymes of the animal tissues (STARKENSTEIN). Inosite is found in plants, especially in the developing organs (MEILLÈRE), and according to STARKENSTEIN[5] it occurs to a greater extent in the organs of young animals as compared with those of older animals. From this it follows that inosite is probably not a decomposition product of metabolism, but rather a body necessary for the development of the cells (MEILLÈRE); but according to STARKENSTEIN the facts are different.

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[1] Maly's Jahresb., **22**.

[2] Bull. soc. chem. (2), **47** and **48**; Compt. Rend., **104**.

[3] Neuberg, Bioch. Zeitschr., **9**; P. Meyer, *ibid.*, **9**.

[4] Hilger, Annal. d. Chem. u. Pharm., **160**; Vohl, Ber. d. d. Chem. Gesellsch., **9**.

[5] Winterstein, Ber. d. d. chem. Gesellsch., **30**; and Zeitschr. f. physiol. chem., **58**; Posternak, Contribution a l'étude chim. de l'assimilation chlorophyllienne. Revue générale botanique, Tome **12** (1900), and Compt. Rend., **137**; Suzuki, Yoshimura and Takaishi, Bull. agric. Univers. Tokio, **7**; Starkenstein, Bioch. Zeitschr., **30**.

According to STARKENSTEIN the free inosite is without importance and is only a decomposition product of metabolism; of importance, especially for young, growing individuals is according to this worker only the phytin, which is decomposed in the intestine by bacteria, and in the tissues by enzymes, and correspondingly supplies phosphoric acid and lime to the organism while the inosite is excreted as a valueless cleavage product. The free inosite in the animal body originates according to STARKENSTEIN from the inositephosphoric acid and in this sense the assumption of ROSENBERGER[1] as to the occurrence of an inositogen in the animal body, is substantiated.

Inosite, which almost without exception is inactive mesoinosite, crystallizes in large, colorless, rhombic crystals of the monoclinic system, or, if not pure and if only a small quantity crystallizes, it forms groups of fine crystals similar to cauliflower. It loses its water of crystallization at 110° C., also if exposed to the air for a long time. Such exposed crystals are non-transparent and milk-white. The crystals melt at 225° C. when dry. Inosite dissolves in 7.5 parts of water at ordinary temperature, and the solution has a sweetish taste. It is insoluble in strong alcohol and in ether. It dissolves cupric hydrate in alkaline solutions, but does not reduce on boiling. It gives negative results with MOORE'S test and with BÖTTGER-ALMÉN'S bismuth test. It does not ferment with beer-yeast, but may undergo lactic- and butyric-acid fermentation. With an excess of nitric acid inosite is oxidized to *rhodizonic acid*, and the following reaction depends upon this.

If inosite is evaporated to dryness on paltinum-foil with nitric acid and the residue treated with ammonia and a drop of calcium chloride solution and carefully re-evaporated to dryness, a beautiful rose-red residue is obtained (SHERER'S inosite test). If we evaporate an inosite solution to incipient dryness and moisten the residue with a little mercuric nitrate solution, there is obtained a yellowish residue on drying which becomes a beautiful red on strongly heating. The coloration disappears on cooling, but it reappears on gently warming (GALLOIS' inosite test). Other inosite reactions have been suggested by DENIGÈS[2] and others.[3]

To prepare inosite from a liquid or from a watery extract of a tissue, the proteins are first removed by coagulation at boiling heat. The filtrate

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[1] Meillère, Journ. d. Chim. et Pharm. (6) 28; Starkenstein, Zeitschr. f. exp. Path. u. Therap. 5, Bioch. Zeitschr. 30 and Zeitschr. f. physiol. Chem. 58; Rosenberger, *ibid.*, 56, 57 and 58.

[2] Compt. rend. soc. biol., 62.

[3] In regard to the salts of phytin and compounds of inosite see Anderson, Journ. of biol. Chem. 11 and 12.

is precipitated by sugar of lead, this filtrate boiled with basic lead acetate and allowed to stand 24–48 hours. The precipitate thus obtained, which contains all the inosite, is decomposed in water by H_2S. The filtrate is strongly concentrated, treated with 2–4 vols. hot alcohol, and the liquid removed as soon as possible from the tough or flaky masses which ordinarily separate. If no crystals separate from the liquid within twenty-four hours, then treat with ether until the liquid has a milky appearance and allow it to stand. In the presence of a sufficient quantity of ether, crystals of inosite separate within twenty-four hours. The crystals thus obtained, as also those which are directly obtained from the alcoholic solution, are recrystallized by redissolving in very little boiling water and adding 2–4 vols. of alcohol. MEILLÈRE [1] and others have suggested modifications in the methods for detecting and quantitatively estimating inosite.

Scyllite is a body which is isomeric with inosite, according to JOH. MÜLLER,[2] and which was found long ago in the kidneys, liver and spleen of Plagiostomata and also in the plant kingdom as *cocosite* and *quercinite*. Scyllite crystallizes in shining prisms, is soluble in water 1:100 at 18° C., is similar to inosite in its reactions, but has a much higher melting-point, namely about 360° C. From the adductor muscles of the Mytilus JANSSEN [3] has isolated a substance, called *mytilite* which is crystalline, soluble with difficulty in cold water and readily soluble in hot water, and having the formula $C_6H_{12}O_6.2H_2O$. He claims that it is stereisometric with the alcohol quercite.

Glycogen is a constant constituent of the living muscle, while it may be absent in the dead muscle. The quantity of glycogen varies in the different muscles of the same animal and according to MAIGNON this is not only true for the same muscles in both halves of the body but also for different parts of the same muscle. BÖHM found 10 p. m. glycogen in the muscles of cats, and moreover he found a smaller amount in the muscles of the extremities than in those of the rump. MOSCATI found an average of 4 p. m. in human muscles, and SCHÖNDORFF [4] has found a maximum of 37.2 p. m. in the dog-muscle. Reports as to the quantity of glycogen in the heart are conflicting; although the heart is considered as somewhat poorer in glycogen than the other muscles, still this difference is not very great, and can be explained by the ready disappearance of glycogen from the heart after death, as well as after starvation and after strong work (BORUTTAU, JENSEN [5]). Work and food have a great influence upon the quantity of glycogen. BÖHM found 1–4 p. m. glycogen in the muscles of fasting animals, and 7–10 p. m. after partak-

[1] Compt. rend. soc. biol., **60**, and Journ. d. Chim. et Pharm. (6), **24**; see also Starkenstein, Zeitschr. f. exp. Path. u. Ther., **5**.

[2] Ber. d. d. chem. Gesellsch., **40**.

[3] Zeitschr. f. physiol. Chem., **85**.

[4] Maignon, Journ. de physiol. et d. path. **10** Böhm, Pflüger's Arch., **23**, 44; Schöndorff, *ibid.*, **99**; Moscati, Hofmeister's Beitrage, **10**.

[5] Boruttau, Zeitschr. f. physiol. Chem., 18; Jensen, *ibid.*, **35**.

ing of food. As stated in Chapter VII, work, starvation, and lack of carbohydrates in the food cause the glycogen to disappear earlier from the liver than from the muscles.

The *sugar of the muscles*, of which only traces occur in the living muscle, and which is probably formed after the death of the muscle from the muscle-glycogen, is, according to the investigations of PANORMOFF, in part glucose, but consists principally of maltose (OSBORNE and ZOBEL[1]) with some dextrin.

Lactic Acids. Of the oxypropionic acids with the formula $C_3H_6O_3$ there is one, ethylene lactic acid, $CH_2(OH).CH_2.COOH$, which is not found in the animal body, and therefore has no physiological chemical interest.

Indeed only α-oxypropionic acid or ethylidene lactic acid, $\begin{matrix}CH_3\\ \dot{C}H(OH)\\ \dot{C}OOH\end{matrix}$, of which there are two physical isomers, namely, the dextrorotatory PARALACTIC OR SARCOLACTIC ACID, and the LEVOLACTIC ACID obtained by SCHARDINGER by the fermentation of cane-sugar by means of a special bacillus. This levolactic acid, which is formed by the typhoid bacillus and various vibriones[2] need not be discussed here, and we will only treat here the *d-l*-lactic acid (the inactive fermentation lactic acid) and the dextrolactic acid.

The *fermentation lactic acid*, which is formed from lactose by allowing milk to sour, and by the acid fermentation of other carbohydrates, is considered to exist in small quantities in the muscles (HEINTZ), in the gray matter of the brain (GSCHEIDLEN), and in diabetic urine. The occurrence of fermentation lactic acid in the brain and other organs is still very improbable and has been disputed by MORIYA.[3] During digestion this acid is also found in the contents of the stomach and intestine, and as alkali lactate in the chyle. The *paralactic acid*, is at all events, the true acid of meat extracts, and this alone has been found with certainty in dead muscle. The lactic acid which is found in the brain, spleen, lymphatic glands, thymus, thyroid gland, blood, bile, pathological transudates, osteomalacial bones, in perspiration in puerperal fever, in the urine after fatiguing marches, in acute yellow atrophy of the liver,

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[1] Panormoff, Zeitschr. f. physiol. Chem., 17; Osborne and Zobel, Journ. of Physiol., 29.

[2] See Schardinger, Monatshefte f. Chem., 11; Blachstein, Arch. des sciences biol. de St. Pétersbourg, 1, 199; Kuprianow, Arch. f. Hygiene, 19, and Gosio, *ibid.*, 21; Herzog and Hörth, Zeitschr. f. physiol. Chem., 60.

[3] Heintz, Annal. d. Chem. u. Pharm., 157, and Gscheidlen, Pflüger's Arch., 8, 171; Moriya, Zeitschrift f. physiol. Chem., 43.

in poisoning by phosphorus, and especially after extirpation of the liver seems to be paralactic acid.

The origin of paralactic acid in the animal organism has been sought by several investigators, who took for basis the researches of GAGLIO, MINKOWSKI, and ARAKI, in a decomposition of protein in the tissues. GAGLIO claims a lactic-acid formation by passing blood through the surviving kidneys and lungs. He also found 0.3–0.5 p. m. lactic acid in the blood of a dog after protein food, and only 0.17–0.21 p. m. after fasting for forty-eight hours. According to MINKOWSKI the quantity of lactic acid eliminated by the urine in animals with extirpated livers is increased with protein food, while the administration of carbohydrates has no effect. ARAKI has also shown that if we produce a scarcity of oxygen in animals (dogs, rabbits, and hens) by poisoning with carbon monoxide, by the inhalation of air deficient in oxygen, or by any other means, a considerable elimination of lactic acid (besides sugar and also often albumin) takes place through the urine, an observation which has been confirmed by SAITO and KATSUYAMA.[1] As a scarcity of oxygen, according to the ordinary statements, produces an increase of the protein catabolism in the body, the increased elimination of lactic acid in these cases must be due in part to an increased protein destruction and in part to a diminished oxidation.

ARAKI has not drawn such a conclusion from his experiments, but he considers the abundant formation of lactic acid to be due to a cleavage of the sugar formed from the glycogen. He found that in all cases where lactic acid and sugar appeared in the urine the quantity of glycogen in the liver and muscles was always diminished. Without denying the possibility of a formation of lactic acid from protein, he states that with lack of oxygen we have to deal with an incomplete combustion of the lactic acid derived by a cleavage of the sugar. Although the abundant formation of lactic acid under these circumstances can be explained in different ways, still there are other conditions which make the formation of lactic acid from proteins very probable. To this belongs the lactic acid formation from alanine, in the liver, as mentioned in a previous chapter, and recently further substantiated by EMBDEN and F. KRAUS.[2]

The carbohydrates are also considered as the mother-substance of the lactic acid, as it is now generally admitted that the cleavage of the

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[1] Gaglio, Arch. f. (Anat. u.) Physiol., 1886; Minkowski., Arch exp. Path, u. Pharm., **21** and **31**; Araki, Zeitschr. f. physiol. Chem., **15**, **16**, **17**, and **19**; Saito and Katsuyama, *ibid.*, **32**.

[2] Neuberg and Langstein, Arch. f. (Anat. u.) Physiol. 1903; Embden and F. Kraus, Bioch Zeitschr. **45**.

sugar in the animal body occurs, or at least can occur, with lactic acid as an intermediary step. The views are indeed different[1] as to the closer mechanism of this cleavage, but there does not exist any doubt that a formation of lactic acid, and in fact paralactic acid, can take place from carbohydrates in the animal body. HOPPE-SEYLER[2] held the view that the formation of lactic acid, in the absence of free oxygen, from glycogen or glucose was probably a function of all living protoplasm and in the anærobic metabolism of the animal cells, according to the investigations of STOKLASA[3] and his collaborators on alcoholic fermentation in the tissues, a formation of alcohol and carbon dioxide takes place from the sugar with lactic acid as intermediary step. The correctness of these statements is now disputed from many sides, but we have direct observations which speak positively for a lactic acid formation from glycogen or sugar. Thus EMBDEN[4] and co-workers have found that on transfusing blood through the liver rich in glycogen, a formation of lactic acid takes place, and an abundance of lactic acid is formed when blood rich in sugar is transfused through a glycogen free liver, while a blood poor in sugar led only to a very inconsiderable formation of lactic acid.

Certain investigators (see page 333) admit of the occurrence of glyceric aldehyde (and also dioxyacetone) as intermediary products in the formation of lactic acid from sugar. Another intermediary product in the lactic acid formation has been shown by recent thorough investigations to be *methylglyoxal*, $CH_3.CO.CHO$. An abundant formation of lactic acid from methylglyoxal has been obtained by certain investigators, such as DAKIN and DUDLEY, and by NEUBERG, in experiments with tissues, organ extracts and organ pulp, and by LEVENE and MEYER[5] in experiments with leucocytes or kidney tissue. The process is of an enzymotic nature and the active enzyme, which also converts phenylglyoxal into mandelic acid has been called *glyoxylase* by DAKIN and DUDLEY. The process is reversible according to these experimenters, in that they have been able to show a retransformation of lactic acid into methylglyoxal. They also found that lactic acid as well as methylglyoxal could form glucose in diabetic animals. The detailed procedure in the cleavage of sugar to lactic acid is still undecided.

The carbohydrates, as well as the proteins, it seems, must be considered as the material from which the lactic acid is formed in the body.

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[1] See Embden und Oppenheimer, Bioch. Zeitschr., **45**; Parnas and Baer, *ibid.*, **41**.

[2] Virchow's Festschrift, also Ber. d. deutsch. chem. Gesellsch., **25**, Referatb., 685.

[3] Simácek, Centralbl. f. Physiol., **17**; Stoklasa, Jelinek, and Cerny, *ibid.*, **16**. In regard to opposed statements see Harden and Mac Lean, Journ. of Physiol., **42**.

[4] Embden and Almagia with F. Kraus, Bioch. Zeitschr. **45**; S. Oppenheimer, *ibid.*, **45**.

[5] Dakin and Dudley, Journ. of biol. Chem., **14**; Neuberg, Bioch. Zeitschr., **49**; Levene and Meyer, Journ. of biol. Chem., **14**.

The phosphocarnic acid (SIEGFRIED) and the inosite are also considered as possible mother-substances for sarcolactic acid. Further research will show whether also other mother-substances for this acid occur. The autolytic experiments of TÜRKEL [1] with livers and the formation of lactic acid in the muscles, not from carbohydrates, inosite or alanine, as observed by EMBDEN [2] and his collaborators seem to indicate this.

The lactic acids are amorphous. They have the appearance of colorless or faintly yellowish, acid-reacting syrups, which mix in all proportions with water, alcohol, or ether. The salts are soluble in water, and most of them also in alcohol. The two acids are differentiated from each other by their different optical properties—paralactic acid being dextrogyrate, while fermentation lactic acid is optically inactive—also by their different solubilities and the different amounts of water of crystallization of the calcium and zinc salts. The zinc salt of fermentation lactic acid dissolves in 58–63 parts of water at 14–15° C., and contains 18.18 per cent water of crystallization, corresponding to the formula, $Zn(C_3H_5O_3)_2+3H_2O$. The zinc salt of paralactic acid dissolves in 17.5 parts of water at the above temperature and contains ordinarily 12.9 per cent water, corresponding to the formula, $Zn(C_3H_5O_3)_2+2H_2O$. The calcium salt of fermentation lactic acid dissolves in 9.5 parts water and contains 29.22 per cent (=5 molecules) water of crystallization, while calcium paralactate dissolves in 12.4 parts water and contains 24.83 or 26.21 per cent (=4 or 4½ molecules) water of crystallization. Both calcium salts crystallize, not unlike tyrosine, in spears or tufts of very fine microscopic needles. HOPPE-SEYLER and ARAKI, who have closely studied the optical properties of the lactic acids and lactates, consider the lithium salt as best suited for the preparation and quantitative estimation of the lactic acids. The lithium salt contains 7.29 per cent Li. For further information as to the salts and specific rotation of the lactic acids see HOPPE-SEYLER-THIERFELDER'S Handbuch, 8. Aufl., 1909.[3]

Lactic acids may be detected in organs and tissues in the following manner: After complete extraction with water, the protein is removed by coagulation at boiling temperature and the addition of a small quantity of sulphuric acid. The liquid is then exactly neutralized, while boiling, with caustic baryta, and then evaporated to a syrup after filtration. The residue is precipitated with absolute alcohol, and the precipitate completely extracted with alcohol. The alcohol is entirely distilled from the united alcoholic extracts, and the neutral residue is

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[1] Türkel, Bioch. Zeitschr., **20**. The statements on the formation of lactic acid in the muscle autolysis are rather conflicting; see Fletcher, Journ. of Physiol., **43**.

[2] Embden, Kalberlah and Engel, Bioch. Zeitschr. **45**; Kondo, *ibid.*, **45**.

[3] See also E. Jungfleisch, Compt. Rend., **139**, **140**, and **142**; Herzog and Slansky, Zeitschr. f. physiol. Chem., **73**.

shaken with ether to remove the fat. The residue is dissolved in water and phosphoric acid is added, and the solution repeatedly shaken with fresh quantities of ether, which dissolves the lactic acid. The ether is now distilled from the united ethereal extracts, the residue dissolved in water, and this solution carefully warmed on the water-bath to remove the last traces of ether and volatile acids. A solution of zinc lactate is prepared from this filtered solution by boiling with zinc carbonate, and this is evaporated until crystallization commences, and is then allowed to stand over sulphuric acid. An analysis of the salts is necessary in careful work. In regard to methods for the detection and quantitative estimation of lactic acid we must refer to larger hand-books.

Fat is never absent in the muscles. Some fat is always found in the intermuscular connective tissue; but the muscle-fibers themselves also contain fat. The quantity of fat in the real muscle substance is always small, usually amounting to about 10 p. m. or somewhat more. A considerable quantity of fat in the muscle-fibers is found only in fatty degeneration. A part of the muscle-fat can be readily extracted, while another part can be extracted only with the greatest difficulty. This latter part, it is claimed, exists finely divided in the contractile substance itself and is richer in free fatty acids, standing, according to ZUNTZ and BOGDANOW,[1] in close relation to the activity of the muscles because it is consumed during work. *Lecithin* is a regular constituent of the muscles, and it is quite possible that the fat which is difficult of extraction and which is rich in fatty acids depends in part on a decomposition of the lecithin and the phosphatides. ERLANDSEN has shown that *phosphatides* of various kinds occur in the muscles, the quantities varying in different muscles. According to him the ox-heart muscle is richer in phosphatides than the muscle of the thigh, and RUBOW[2] claims that the heart of the dog is richer in phosphatides than the striated muscle. ERLANDSEN found lecithin and diamino-phosphatide in the heart as well as the thigh-muscle, while the monoamido-phosphatide cuorin, which occurs abundantly in the heart, is found as traces in the thigh-muscle. COSTANTINO[3] has carried on investigations on the division of the inorganic and organic phosphorus in striated and smooth muscles.

The Mineral Bodies of the Muscles. The ash remaining after burning the muscle, which amounts to about 10–15 p. m., calculated on the moist muscle, is acid in reaction. The largest constituent of the ash is potassium, whose occurrence, according to MACALLUM,[4] is restricted to the dark

[1] Arch. f. (Anat. u.) Physiol., 1897.

[2] Erlandsen, Zeitschr. f. physiol. Chem., **51**; Rubow, Arch. f. exp. Path. u. Pharm., **52**.

[3] Bioch. Zeitschr., **43**.

[4] Journ. of Physiol., **32**.

diagonal bundles, and phosphoric acid. Next in amount we have sodium and magnesium, and lastly calcium, chlorine, and iron oxide. Sulphates exist only as traces in the muscles, but are formed by the burning of the proteins of the muscles, and therefore occur in abundant quantities in the ash. The muscles contain such a large quantity of potassium and phosphoric acid, that potassium phosphate seems to be, unquestionably, the predominating salt. Chlorine is found in such insignificant quantities that it is perhaps derived from a contamination with blood or lymph. The quantity of magnesium is, as a rule, considerably greater than that of calcium. Iron occurs only in very small amounts. The water of the muscle occurs in part free and partly as imbibition water of the colloids. According to the investigations of JENSEN and FISCHER[1] only a small part, a few per cent, of the total water exists in this condition.

URANO[2] has removed the salts of the intermediary fluid (blood, lymph) from frogs' muscles by treating them with an isotonic cane-sugar solution (of 6 per cent) and in this manner found that the sodium did not belong to the muscle substance itself, but to the intermediary fluid, while at least a small part of the chlorine is a true muscle constituent. He also calculated, from the quantity of sodium, that the intermediary fluid, if it has about the same composition as the muscle plasma, makes up about one-sixth of the volume of the muscle. According to further investigations of URANO the possibility of a disturbance in the osmotic properties of the muscle-fibers by the sugar solution is not entirely excluded, and the question whether the muscle-fibers are free from sodium or not has therefore not been positively decided. FAHR'S[3] researches make the absence of sodium in frog's muscle very probable.

The importance of the various mineral bodies for the function of the muscles has been the subject of numerous investigations and by many of these we have obtained further proof, as mentioned in a previous chapter, of the ion action of the electrolytes and the antagonism of different ions. These researches also indicate that each of the ions Na, Ca, and K plays a certain part in the maintenance of the excitability, in the contraction and in the fatigue of the muscle (heart); still these investigations have not led to concordant results, so that we are not yet clear as to the action of these ions. Nevertheless it seems to be established that the combined action of various ions is a necessity for the normal function of the muscles. It has also been shown that it is possible to maintain the muscle (the heart) in regular activity for a long time by means of a transfusion of liquid saturated with oxygen, and which con-

[1] Jensen and Fischer, Bioch. Zeitschr., **20**.
[2] Zeitschr. f. Biol., **50**.
[3] Urano, *ibid.*, **51**; Fahr., *ibid.*, **52**.

tained about 7 p. m. NaCl, besides small amounts of $CaCl_2$ (0.2 p. m.), KCl (0.1 p. m.), and $NaHCO_3$ (0.1 p. m.).

The *gases* of the muscles consist of large quantities of carbon dioxide besides traces of nitrogen.

In regard to the permeability of the muscles for various bodies there are the complete investigations of OVERTON.[1] The different sheaths of the muscles, the sarcolemma and perimysium internum, offer no very great resistance to the diffusion of the most soluble crystalloid compounds, while the muscle-fibers, on the contrary (exclusive of the sarcolemma), are almost if not entirely impervious to most inorganic compounds and to many organic compounds. The muscle-fibers themselves are actually semipermeable structures which are permeable to water but not to the molecules or ions of sodium chloride and of potassium phosphate. The muscle-fibers, as well as the various sheaths, are impermeable to colloids.

The behavior of the numerous bodies investigated cannot be discussed in this work. The general rule is as follows: All compounds which, besides having a marked solubility in water, are readily soluble in ethyl ether, in the higher alcohols, in olive-oil and in similar organic solvents, or are not much less soluble in the last-mentioned solvents than in water, pass through the living muscle-fibers with great ease. The greater the difference between the solubility of a compound in water and in the other solvents mentioned, the slower does the passage into the muscle-fibers take place. The permeability changes essentially on the death of the muscle.

The living muscle-fibers are readily permeable to oxygen, carbon dioxide, and ammonia, while the hexoses and disaccharides do not readily pass into them. It is very remarkable that a great portion of those compounds which take part in the normal metabolism of plants and animals belongs to those bodies to which the muscle-fibers (and also other cells) are entirely or at least nearly impermeable. On the contrary, derivatives can be prepared from these bodies which pass into the cells very readily, and OVERTON finds that it is not impossible that the organism in part makes use of a similar artifice in order to regulate the concentration of the nutritive bodies within the protoplasm. (See Chapter I.)

Rigor Mortis of the Muscles. If the influence of the circulating oxygenated blood is removed from the muscles, as after the death of the animal or by ligature of the aorta or the muscle-arteries (STENSON'S test), *rigor mortis* sooner or later takes place. The ordinary rigor appearing under these circumstances is called the spontaneous or the

[1] Pflüger's Arch., **92**. See also Höber, *ibid.*, **106**, and Hamburger, Osmotischer Druck und Ionenlehre. Bd. **3**.

fermentative rigor, because it seems to depend in part on the action of an enzyme. A muscle may also become stiff or other reasons. The muscles may become momentarily stiff by warming, in the case of frogs to 40°, in mammalia to 48–50°, and in birds to 53° C. Distilled water may also produce a rigor in the muscles (water-rigor). Acids, even very weak ones, such as carbon dioxide, may quickly produce a rigor (acid-rigor), or hasten its appearance. A number of chemically different substances, such as chloroform, ether, alcohol, ethereal oils, caffeine, and many alkaloids, produce a similar effect.

When the muscle passes into *rigor mortis* it becomes shorter and thicker, harder and non-transparent, and less ductile. The acid part of the amphoteric reaction becomes stronger; which is explained by most investigators by the assumption of a formation of lactic acid. There is hardly any doubt that this increase in acidity may at least in part be due to a transformation of a part of the diphosphate into monophosphate by the lactic acid. The statements as to whether in the rigor mortis muscles, besides acid phosphate also free lactic acid exists or not are rather contradictory;[1] that an acid formation precedes the rigor is generally admitted and this acid formation is now accepted as being in close relation to the rigor. While we used to consider the appearance of a clot consisting of myosin (KÜHNE) or cf myogen- and myosin fibrin (v. FÜRTH) as the essential moment for the rigor, we now admit, based upon the investigations of MEIGS, v. FÜRTH and LENK,[2] that the most essential factor is the imbibition of the disdiaclasts, which become broader or shorter, by their taking up of water from the sarcolemma fluid and this action produced by the acid formation. This view stands in accord with the experience on the imbibition of colloids and muscles in water or salt solutions, in the presence and absence of acid, as well as the fact that the rigor can be retarded by the artificial circulation of blood or by the action of salt solutions, namely by those which contain small amounts of $NaHCO_3$. This also agrees well with the old experience, that the muscle work, which is also connected with a formation of acid, accelerates the appearance of rigor.

On further post-mortal changes, namely by a further accumulation of acid, a progressive coagulation of the proteins gradually occurs. In this coagulation the ability of the colloid systems to imbibe water

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[1] It is impossible to enter into the details of the disputed theories as to the reaction of the muscles, etc. We shall only refer to the works of Röhmann, Pflüger's Arch., **50** and **55**, and Heffter, Arch. f. exp. Path. u. Pharm., **31** and **38**. These works contain also the researches of the earlier investigators more or less completely.

[2] Meigs, Journ. of Physiol. **39** and especially, Amer. Journ. of Physiol., **24** and **26**; v. Fürth and Lenk, Bioch. Zeitschr., **33**, and Wien. klin. Wochenschr., **24** (1911).

diminishes, water is given off, and a re-imbibition takes place and the so-called " solution of the rigor " appears (v. FÜRTH and LENK).

The ordinary rigor is an *acid rigor* and the same applies, according to MEIGS,[1] to the *water rigor* as a shortening of the muscles takes place when placed in distilled water, by a formation of lactic acid, and because when such a muscle is placed in RINGER'S solution the acid is removed and the muscle again expands.

The views are rather contradictory in regard to the production of *heat rigor*. According to v. FÜRTH this rigor depends upon the coagulation of certain proteins, and its occurrence at lower temperatures in cold-blooded as compared with warm-blooded animals is due, according to v. FÜRTH, to the fact that in the first a soluble myogen fibrin occurs preformed in the muscle which coagulates at 30–40° C., while in the warm-blooded animals the coagulating substance is musculin (myosin of v. FÜRTH) which coagulates at a higher temperature. According to INAGAKI[2] the various stages in contractions occurring on heating a muscle (frog) do not correspond to those of the coagulation of the protein which would occur on heating the muscle plasma, and MEIGS has arrived at a similar view. It must be remarked that also a lactic acid formation takes place on heating a muscle, and this prevents an exact comparison of the coagulation of the proteins within and outside of the muscle. The observations of VERNON that the striated and the smooth muscles on heating to between 40 and 50° behave differently, in that the striated become shorter and the smooth become longer, while both kinds become shorter at higher temperatures, indicates against a coagulation at these low temperatures. According to MEIGS[3] we must here also admit of an *imbibition rigor*, due to the formation of lactic acid, and the different behavior of the two kinds of muscle depends upon a different arrangement of their anatomical elements.

The *chemical rigor* produced by different chemically active substances is also produced, according to MEIGS as well as to v. FÜRTH and LENK, upon a formation of acid, causing a chemical damage of the muscles, and is to be considered as an imbibition rigor.

As it is now generally admitted that the formation of lactic acid during the death of the muscle is the cause of the muscle rigor, the question arises, from what constituents of the muscle is this acid derived? The most probable explanation is that the lactic acid is produced from the glycogen, as certain investigators, such as NASSE and WERTHER, have observed a decrease in the quantity of glycogen in rigor of the muscle.

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[1] Journ. of Physiol., **39**.

[2] Inagaki, Zeitschr. f. Biol., **48**; Meigs, Journ. of Physiol., **24**.

[3] Vernon, Journ. of Physiol., **24**; Meigs, Amer. Journ. of Physiol., **24**.

On the other side, EÖHM has observed cases in which no consumption of glycogen took place in rigor of the muscle, and he also found that the quantity of lactic acid produced is not proportional to the quantity of glycogen. According to MOSCATI [1] the diminution in the glycogen is independent of the appearance of rigor. It is therefore possible that the consumption of glycogen and the formation of lactic acid in the muscles are two processes independent of each other, and, as above stated in regard to the formation of paralactic acid, the origin of the lactic acid in the muscle is still not positively known. The phosphocarnic acid must also be considered as a mother-substance of the lactic acid, and of the carbon dioxide, also formed in the rigor, as it yields lactic acid as well as carbon dioxide on its cleavage.

Metabolism in the Inactive and Active Muscles. It is admitted by a number of prominent investigators, PFLÜGER and COLASANTI, ZUNTZ and RÖHRIG [2], and others, that the metabolism in the muscles is regulated by the nervous system. When at rest, when there is no mechanical exertion, there exists a condition which ZUNTZ and RÖHRIG have designated "*chemical tonus.*" This tonus seems to be a reflex tonus, for it may be reduced by discontinuing the connection between the muscles and the central organ of the nervous system by cutting through the spinal cord or the muscle-nerves. The possibility of reducing the chemical tonus of the muscles in various ways offers an important means of deciding the extent and kind of chemical processes going on in the muscles when at rest. In comparative chemical investigation of the processes in the active and the inactive muscles several methods of procedure have been adopted. The same active and inactive muscles have been compared after removal, also the arterial and venous muscle-blood in rest and activity, and lastly the total exchange of material, the receipts and expenditures of the organism, have been investigated under these two conditions.

By investigations according to these several methods it was found that the resting muscle takes up oxygen from the blood and returns to it carbon dioxide, and also that the quantity of oxygen taken up is greater than the oxygen contained in the carbon dioxide eliminated at the same time. The muscle, therefore, holds in some form of combination a part of the oxygen taken up while at rest. During activity the exchange of material in the muscle, and therewith the exchange of gas, is increased.

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[1] Nasse, Beitr. z. Physiol. der kontrakt. Substanz, Pflüger's Arch., **2**; Werther, *ibid.*, **46**; Böhm, *ibid.*, **23** and **46**; Moscati, Hofmeister's Beiträge, **10**.

[2] See the works of Pflüger and his pupils in Pflüger's Arch., **4**, **12**, **14**, **16**, and **18**; Röhrig, *ibid.*, **4**. See also Zuntz, *ibid.*, **12**. In regard to the metabolism after curare poisoning, see also Frank and Voit, Zeitschr. f. Biologie, **42**, and Frank and Gebhard, *ibid.*, **43**.

The animal organism takes up much more oxygen in activity than when at rest, and eliminates also considerably more carbon dioxide. The quantity of oxygen which leaves the body as carbon dioxide during activity is much larger than the quantity of oxygen taken up at the same time; and the venous muscle-blood is poorer in oxygen and richer in carbon dioxide during activity than during rest. The exchange of gases in the muscles during activity is the reverse of that at rest, for the active muscle gives up a quantity of carbon dioxide which does not correspond to the quantity of oxygen taken up, but is considerably greater. It follows from this that in muscular activity not only does oxidation take place, but also splitting processes occur. This also results from the fact that removed blood-free muscles when placed in an atmosphere devoid of oxygen can labor for some time and still yield carbon dioxide (HERMANN[1]).

During muscular inactivity, in the ordinary sense, a consumption of glycogen takes place. This is inferred from the observations of several investigators, that the quantity of glycogen is increased and its corresponding consumption reduced in those muscles whose chemical tonus is reduced either by cutting through the nerve or for other reasons (BERNARD, CHANDELON, VAY,[2] and others). In activity this consumption of glycogen is increased, and it has been positively proved by the researches of numerous investigators[3] that the quantity of glycogen in the muscles in activity decreases quickly and freely. The sugar is removed from the blood and consumed during activity.[4] The recent investigations of JOH. MÜLLER, LOCKE and ROSENHEIM and CAMIS[5] have given direct proof of the consumption of sugar during muscular activity. In experiments on surviving hearts of different animals through which was perfused a salt solution containing sugar, they could detect an undoubted consumption of sugar which was quite considerable and which to all appearances was used as material for muscle work.

The amphoteric reaction of the inactive muscles is changed during

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[1] L. Hermann, Unters. über d. Stoffwechsel der Muskeln, etc., Berlin, 1867. In regard to gas exchange in removed muscles, see also J. Tissot, Arch. de Physiol. (5), **6** and **7**, and Compt. Rend., **120**.

[2] Chandelon, Pflüger's Arch., **13**; Vay, Arch. f. exp. Path. u. Pharm., **34**, which also contains the pertinent literature.

[3] Nasse, Pflüger's Arch., **2**; Weiss, Wien. Sitzungsber., **64**; Külz, in Ludwig's Festschrift, Marburg, 1890; Marcuse, Pflüger's Arch., **39**; Manché, Zeitschr. f. Biologie, **25**; Morat and Dufour, Arch. de Physiol. (5), **4**.

[4] Chauveau and Kaufmann, Compt. Rend., **103**, **104**, and **105**; Quinquaud, Maly's Jahresber., **16**; Morat and Dufour, l. c.: Cavazzani, Centralbl. f. Physiol., **8**; Seegen, "Die Zuckerbildung im Thierkörper," Berlin, 1890, Centralbl. f. Physiol., **8**, **9**, and **10**; Arch. f. (Anat. u.) Physiol. 1895 and 1896; Pflüger's Arch., **50**.

[5] Joh. Müller, Zeitschr. f allgem. Physiol., **3**; Camis, *ibid.*, **8**; Locke and Rosenheim, Journ. of Physiol., **26**.

activity to an acid reaction (DU BOIS-REYMOND and others), and the acid reaction increases, to a certain point, with the work. The quickly contracting pale muscles produce, according to GLEISS,[1] more acid during activity than the more slowly contracting red muscles. Numerous investigations have been carried out on the cause of this increased acid reaction, using the muscles *in situ* and also upon removed muscles and rather contradictory results have been obtained. Some have found a diminution in the amount of lactic acid in the active muscle while others have found an increase.[2] The work of FLETCHER and HOPKINS[3] is of great importance in this disputed question, in which they show that in the removal of the muscle, and in its preparation for the testing for lactic acid several sources of error are possible. The mechanical irritation as well as warming or treating the muscle with alcohol (not ice-cold) can lead to a formation of lactic acid. It was also shown that the absence of oxygen accelerated the formation or accumulation of lactic acid, while an abundance of oxygen had the opposite effect.

It is evident that the experiments with the muscles *in situ*—in other words, with muscles through which blood is passing—cannot yield any conclusion to the above question, as the lactic acid formed during work may perhaps be removed by the blood. The following objections can be made against those experiments in which lactic acid has been found, after moderate work, in the blood or the urine, as also especially against the experiments with removed active muscles, namely, that in these cases the supply of oxygen to the muscles was not sufficient, and that the lactic acid formed thereby is not, in accordance with the views of HOPPE-SEYLER, a perfectly normal process. The same is probably true also for the formation of lactic acid with excessive work during life, and ZILLESSEN[4] has found that the artificial cutting off of the oxygen supply in the muscles during life, that more lactic acid was formed than under normal conditions. Other observations indicate a formation of lactic acid during activity. Thus SPIRO and recently also H. FRIES[5] found an increase in the quantity of lactic acid in the blood during work. COLASANTI and MOSCATELLI found small quantities of lactic acid in human urine after strenuous marches, and WERTHER[6] observed an abundance of lactic acid in the urine of frogs after tetanization.

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[1] Pflüger's Arch., 41.

[2] Astaschewsky, Zeitschr. f. physiol. Chem., 4; Warren, Pflüger's Arch., 24, Monari, Maly's Jahresber., 19; Heffter, Arch. f. exp. Path. u. Pharm., 31; Marcuse, l. c.; Werther, Pflüger's Arch., 46; Spiro, Zeitschr. f. physiol. Chem., 1; Colasanti.

[3] Journ. of Physiol., 25.

[4] Hoppe-Seyler, l. c. and Zeitschr. f. physiol. Chem., 19, 476; Zillessen, *ibid.*, 15.

[5] Spiro, Zeitschr. f. physiol. Chem. 1; Fries, Bioch. Zeitschr., 35.

[6] Colasanti and Moscatelli; Maly's Jahresb.. 17, 212; Werther, Pflüger's Arch., 46.

According to SIEGFRIED the amount of phosphocarnic acid is diminished during activity. MACLEOD[1] claims that this is true only for intense muscular activity, and the mother-substance of lactic acid can at least in part be phosphocarnic acid. The question as to the formation of lactic acid during activity, and the origin of the phosphocarnic acid is certainly in many points somewhat undecided; the general view seems to be, that during work lactic acid is formed, which transforms a part of the diphosphates into monophosphates.

The amount of proteins in the removed muscles is, according to the earlier investigators, decreased by work. The correctness of this statement is, however, disputed by other investigators. Earlier reports in regard to the nitrogenous extractive bodies of the muscle in rest and in activity are likewise uncertain. According to the recent researches of MONARI[2] the total quantity of creatine and creatinine is increased by work, and indeed the amount of creatinine is especially augmented by an excess of muscular activity. The creatinine is formed essentially from the creatine. The investigations of GRAHAM BROWN and CATHCART on removed nerve-muscle preparations of frogs, and those of S. WEBER[3] on hearts, indicate an increase in the formation of creatine and creatinine during work. WEBER found that the working heart gave up creatine (and creatinine) to RINGER'S solution, and indeed much more when strongly active than during a lesser activity. An increased creatinine elimination after work does not occur according to several investigators (see Chapter XIV) and according to PEKELHARING and v. HOOGENHUYZE with ordinary muscle activity neither an increased creatine formation nor an increased creatinine elimination takes place. In the tonic contraction the creatine is formed from the proteins, and correspondingly according to PEKELHARING and HARKINK[4] the creatinine elimination is increased under the influence of the muscle tonus. The purine bases are produced, according to BURIAN, in the muscles themselves, also in activity, and an increased formation takes place during work due to a re-formation. SCAFFIDI[5] found on the contrary, with frogs and tortoise, during work that a diminution of the total quantity of purine bases occurred and indeed not the free but the combined purines.

Attempts have been made to solve the question relative to the behavior of the nitrogenized constituents of the muscle at rest and during

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[1] Siegfried, Zeitschr. f. physiol. Chem., **21**; Macleod, *ibid.*, **28**.

[2] Maly's Jahresber., **19**, 296.

[3] Cathcart and Graham Brown, Journ. of Physiol., **37**; Weber, Arch. f. exp. Path. u. Pharm., **58**.

[4] Pekelharing and v. Hoogenhuyze, Zeitschr. f. physiol. Chem., **64**, with Harkink, *ibid.*, **75**.

[5] Burian, Zeitschr. f. physiol. Chem., **43**; Scaffidi; Bioch. Zeitschr., **30**.

activity by determining the total quantity of nitrogen eliminated under these different conditions of the body. While formerly it was held with LIEBIG that the elimination of nitrogen by the urine was increased by muscular work, the researches of several experimenters, especially those of VOIT on dogs, and PETTENKOFER and VOIT on men, have led to quite different results. They have shown, as has also lately been confirmed by other investigators, especially I. MUNK and HIRSCHFELD,[1] that during work no increase, or only a very insignificant increase, in the elimination of nitrogen takes place.

We should not omit to mention the fact that a series of experiments has been made showing a significant increase in the metabolism of proteins during or after work. There are for example the observations of FLINT and of PAVY on a pedestrian, v. WOLFF, v. FUNKE, KREUZHAGE, and KELLNER on a horse, and DUNLOP and his collaborators on working human beings, and of KRUMMACHER, PFLÜGER, ZUNTZ and his pupils,[2] and others. The researches on the elimination of sulphur during rest and activity also belong to this category. The elimination of nitrogen and sulphur runs parallel with the metabolism of proteins in resting and active persons, and the quantity of sulphur excreted by the urine is therefore also a measure of the protein catabolism. The earlier researches of ENGELMANN, FLINT, and PAVY, as well as the more recent ones of BECK and BENEDICT,[3] and DUNLOP and his collaborators, show an increased elimination of sulphur during or after work, and this indicates an increased protein metabolism because of muscular activity.

That an increased destruction of protein is not necessarily produced by work follows from the observations of CASPARI, BORNSTEIN, KAUP, WAIT, A. LOEWY, ATWATER and BENEDICT,[4] that a retention of nitrogen and a deposition of protein occur during work. The discordant observations on the protein destruction during, and caused by, work are not directly in opposition to each other, because the extent of protein metabolism is dependent upon many conditions, such as the quantity

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[1] Voit, Untersuchungen über den Einfluss des Kochsalzes, des Kaffees und der Muskelbewegungen auf den Stoffwechsel (München, 1860), and Zeitschr. f. Biologie, **2**; J. Munk, Arch. f. (Anat. u.) Physiol., 1890 and 1896; Hirschfeld, Virchow's Arch., **121**.

[2] Flint, Journ. of Anat. and Physiol., **11** and **12**; Pavy, The Lancet, 1876 and 1877; v. Wolff, v. Funke, Kellner, cited from Voit, Hermann's Handb., **86**, 197; Dunlop Noel-Paton, Stockman, and Maccadam, Journ. of Physiol., **22**; Krummacher, Zeitschr. f. Biologie, **33**; Pflüger, Pflüger's Arch., **50**; Zuntz, Arch. f. (Anat. u.) Physiol., 1894.

[3] Engelmann, Arch. f. (Anat. u.) Physiol., 1871; Beck and Benedict, Pflüger's Arch., **54**, and also footnote 2.

[4] Caspari, Pflüger's Arch., **33**; Bornstein, *ibid.*; Kaup, Zeitschr. f. Biologie, **43**; Wait, U. S. Depart. Agricult. Bulletin, **89**; (1901) Atwater and Benedict, *ibid.*, Bull., **69** (1899); Loewy, Arch. f. (Anat. u.) Physiol., 1901.

and composition of the food, the condition of the adipose tissue of the body, the action of the work upon the respiratory mechanism, etc., all of which have an influence on the results of the experiments.

What has been said above in regard to the protein catabolism during muscular activity only applies for the metabolism experiments carried on in the generally accepted manner. THOMAS[1] has made an experiment, under RUBNER'S direction, on the action of work upon the nitrogen elimination upon a person when the nitrogen minimum was reduced to the wear and tear quota (see Chapter XVII), and this experiment seems to indicate a small increase in the nitrogen elimination due to work.

The older investigations on the amount of fat in muscles removed after activity and after rest have not led to any definite results. According to the investigations of ZUNTZ and BOGDANOW,[2] the fat belonging to the muscle-fibers, which is extracted with difficulty, takes part in work. Besides these there are several researches by VOIT, PETTENKOFER and VOIT, J. FRENTZEL,[3] and others which make an increased destruction of fat during work probable or proved.

If the results of the investigations thus far made of the chemical processes going on in the active and inactive muscles were collected, we would find the following characteristics for the active muscle: The active muscle takes up more oxygen and gives off more carbon dioxide than the inactive muscle; still the elimination of carbon dioxide is increased considerably more than the absorption of oxygen. The respiratory quotient, $\frac{CO_2}{O}$, is found to be regularly raised during work; yet this rise, which will be explained in detail in a following chapter on metabolism, can hardly be conditioned on the kind of processes going on in the muscle during activity with a sufficient supply of oxygen. In work a consumption of carbohydrates, glycogen, and sugar takes place. The acid reaction of the muscle becomes greater with work. In regard to the extent of a re-formation of lactic acid opinion is divided. An increased consumption of fat has occasionally been observed. On the behavior of creatine (or creatinine) and purine bodies the statements are somewhat divergent. Protein metabolism has been found increased in certain series of experiments and not in others; but an increased elimination of nitrogen as a direct consequence of muscular exertion has thus far not been positively proved.

In close connection with the above-mentioned facts there is the

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[1] Arch. f. (Anat. u.) Physiol. 1910, Supplelbd.

[2] *Ibid.*, 1897.

[3] Pflüger's Arch., 68.

question as to the material basis of muscular activity so far as it has its origin in chemical processes. In the past the generally accepted opinion was that of LIEBIG, that the source of muscular action consisted of a catabolism of the protein bodies; to-day another generally accepted view prevails. FICK and WISLICENUS [1] climbed the Faulhorn and calculated the amount of mechanical force expended in the attempt. With this they compared the mechanical equivalent transformed in the same time from the proteins, calculated from the nitrogen eliminated in the urine, and found that the work really performed was not by any means compensated by the consumption of protein. It was, therefore, proved by this that proteins alone cannot be the source of muscular activity and that this depends in great measure on the metabolism of non-nitrogenous substances. Many other observations have led to the same result, especially the experiments of VOIT, of PETTENKOFER and VOIT, and of other investigators, whose observations show that while the elimination of nitrogen remains unchanged, the elimination of carbon dioxide during work is very considerably increased. It is also generally considered as positively proved that muscular work is produced, at least in greatest part, by the catabolism of non-nitrogenous substances. Nevertheless there is no warrant for the statement that muscular activity is produced entirely at the cost of the non-nitrogenous substances, and that the protein bodies are without importance as a source of energy.

The investigations of PFLÜGER [2] are of great interest in this connection. He fed a bulldog for more than seven months with meat which alone did not contain sufficient fat and carbohydrates even for the production of heart activity, and then let him work very hard for periods of 14, 35, and 41 days. The positive result obtained by these series of experiments was that "complete muscular activity may be effected to the greatest extent in the absence of fat and carbohydrates," and the ability of proteins to serve as a source of muscular energy cannot be denied.

The nitrogenous as well as the non-nitrogenous nutriments may serve as a source of energy; but the views are divided in regard to the relative value of these. PFLÜGER claims that no muscular work takes place without a decomposition of protein, and the living cell-substance prefers always the protein and rejects the fat and sugar, contenting itself with these only when proteins are absent. Other investigators, on the contrary, believe that the muscles first draw on the supply of non-nitrogenous

[1] Vierteljahrsschr. d. Zürich. naturf. Gesellsch., 10, cited from Centralbl. f. d. med. Wiss., 1866, 309.

[2] Pflüger's Arch., 50.

tained about 7 p. m. NaCl, besides small amounts of $CaCl_2$ (0.2 p. m.), KCl (0.1 p. m.), and $NaHCO_3$ (0.1 p. m.).

The *gases* of the muscles consist of large quantities of carbon dioxide besides traces of nitrogen.

In regard to the permeability of the muscles for various bodies there are the complete investigations of OVERTON.[1] The different sheaths of the muscles, the sarcolemma and perimysium internum, offer no very great resistance to the diffusion of the most soluble crystalloid compounds, while the muscle-fibers, on the contrary (exclusive of the sarcolemma), are almost if not entirely impervious to most inorganic compounds and to many organic compounds. The muscle-fibers themselves are actually semipermeable structures which are permeable to water but not to the molecules or ions of sodium chloride and of potassium phosphate. The muscle-fibers, as well as the various sheaths, are impermeable to colloids.

The behavior of the numerous bodies investigated cannot be discussed in this work. The general rule is as follows: All compounds which, besides having a marked solubility in water, are readily soluble in ethyl ether, in the higher alcohols, in olive-oil and in similar organic solvents, or are not much less soluble in the last-mentioned solvents than in water, pass through the living muscle-fibers with great ease. The greater the difference between the solubility of a compound in water and in the other solvents mentioned, the slower does the passage into the muscle-fibers take place. The permeability changes essentially on the death of the muscle.

The living muscle-fibers are readily permeable to oxygen, carbon dioxide, and ammonia, while the hexoses and disaccharides do not readily pass into them. It is very remarkable that a great portion of those compounds which take part in the normal metabolism of plants and animals belongs to those bodies to which the muscle-fibers (and also other cells) are entirely or at least nearly impermeable. On the contrary, derivatives can be prepared from these bodies which pass into the cells very readily, and OVERTON finds that it is not impossible that the organism in part makes use of a similar artifice in order to regulate the concentration of the nutritive bodies within the protoplasm. (See Chapter I.)

Rigor Mortis of the Muscles. If the influence of the circulating oxygenated blood is removed from the muscles, as after the death of the animal or by ligature of the aorta or the muscle-arteries (STENSON'S test), *rigor mortis* sooner or later takes place. The ordinary rigor appearing under these circumstances is called the spontaneous or the

[1] Pflüger's Arch., **92**. See also Höber, *ibid.*, **106**, and Hamburger, Osmotischer Druck und Ionenlehre. Bd. **3**.

fermentative rigor, because it seems to depend in part on the action of an enzyme. A muscle may also become stiff or other reasons. The muscles may become momentarily stiff by warming, in the case of frogs to 40°, in mammalia to 48–50°, and in birds to 53° C. Distilled water may also produce a rigor in the muscles (water-rigor). Acids, even very weak ones, such as carbon dioxide, may quickly produce a rigor (acid-rigor), or hasten its appearance. A number of chemically different substances, such as chloroform, ether, alcohol, ethereal oils, caffeine, and many alkaloids, produce a similar effect.

When the muscle passes into *rigor mortis* it becomes shorter and thicker, harder and non-transparent, and less ductile. The acid part of the amphoteric reaction becomes stronger; which is explained by most investigators by the assumption of a formation of lactic acid. There is hardly any doubt that this increase in acidity may at least in part be due to a transformation of a part of the diphosphate into monophosphate by the lactic acid. The statements as to whether in the rigor mortis muscles, besides acid phosphate also free lactic acid exists or not are rather contradictory;[1] that an acid formation precedes the rigor is generally admitted and this acid formation is now accepted as being in close relation to the rigor. While we used to consider the appearance of a clot consisting of myosin (KÜHNE) or cf myogen- and myosin fibrin (v. FÜRTH) as the essential moment for the rigor, we now admit, based upon the investigations of MEIGS, v. FÜRTH and LENK,[2] that the most essential factor is the imbibition of the disdiaclasts, which become broader or shorter, by their taking up of water from the sarcolemma fluid and this action produced by the acid formation. This view stands in accord with the experience on the imbibition of colloids and muscles in water or salt solutions, in the presence and absence of acid, as well as the fact that the rigor can be retarded by the artificial circulation of blood or by the action of salt solutions, namely by those which contain small amounts of $NaHCO_3$. This also agrees well with the old experience, that the muscle work, which is also connected with a formation of acid, accelerates the appearance of rigor.

On further post-mortal changes, namely by a further accumulation of acid, a progressive coagulation of the proteins gradually occurs. In this coagulation the ability of the colloid systems to imbibe water

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CHAPTER XI.

BRAIN AND NERVES.

On account of the difficulty in making a mechanical separation and isolation of the different tissue-elements of the central nervous organ and the nerves, we must resort to a few microchemical reactions, principally to qualitative and quantitative investigations of the different parts of the brain, in order to study the varied chemical composition of the cells and the nerve-axes. This study is accompanied with the greatest difficulty, and although our knowledge of the chemical composition of the brain and nerves has been somewhat extended by the investigations of modern times, still it must be admitted that this subject is as yet one of the most obscure and complicated in physiological chemistry.

Proteins of different kinds have been shown to be chemical constituents of the brain and nerves, and these are representatives of the same chief groups as occur in the protoplasm. In the brain there occur some proteins which are insoluble in water and neutral salt solutions, and which resemble the stroma substances of the muscles and cells, while other proteins are soluble in water and neutral salt solutions. Among the latter we find mainly *nucleoproteins* and *globulins*. The nucleoprotein found by Halliburton and also by Levene [1] in the gray substance contains 0.5 per cent phosphorus and coagulates as 55–60°. Levene obtained adenine and guanine but no hypoxanthine as cleavage products. According to Halliburton there are two globulins, namely, the neuroglobulin α, which coagulates at 47°, or as in the case of birds, 50–53°, and the neuroglobulin β, whose coagulation temperature is 70–75°, but which varies somewhat in different animals. In the frog still another protein body occurs, which coagulates at a still lower temperature, about 40°. It must be remarked that the coagulation temperature of α-globulin corresponds with the temperature of the first heat contraction of the nerves of different classes of animals (Halliburton).

[1] Halliburton, On the Chemical Physiology of the Animal's Cell, King's College, London, Physiological Laboratory, Collected Papers No. 1, 1893, and Ergebnisse der Physiologie, 4; Levene, Arch. of Neurology and Psychopathology, 2 (1899).

The gray substance is only slightly richer in proteins than the white substance; but as the *neurokeratin*, which forms the neurolgia, and as a double sheath envelops the outside of the nerves, belongs in great part, or according to KOCH, entirely, to the white substance (KÜHNE, and CHITTENDEN, BAUMSTARK [1]), the gray substance is actually richer in protein. The same is true also for the nucleoprotein or at least for the *nucleins* which v. JAKSCH found in large amounts in the gray substance. The mixture of amino-acids obtained from the proteins of the gray and white substances has about the same composition (ABDERHALDEN and WEIL [2]). Glycocoll could not be detected in this mixture.

The so-called *protagon* has been considered as one of the chief constituents, perhaps the only constituent (BAUMSTARK), of the white substance. This protagon, according to most investigators, is only a mixture of phosphatides with *cerebron* or with a mixture of cerebrosides (see below). Protagon belongs to the so-called brain lipoids, which include three chief groups, *phosphatides*, *cerebrosides* and *cholesterin* and which are contained to a greater extent in the white than in the gray substance. Among the closely studied phosphatides the *cephalin* seems to occur to the greatest extent in the brain. The *lecithin*, according to FRÄNKEL,[3] does not occur in the human brain and only in very small quantities in other brains (of sheep and beef). Other brain phosphatides especially described by THUDICHUM and by FRÄNKEL,[4] have not been positively proved as chemical individuals. The same is true for the *jecorin* and the *sulphurized lipoids* isolated from the human brain and from ox brains. *Cholesterin* occurs chiefly in the white substance. *Fatty acids* and *neutral fats* may be prepared from the brain and nerves; but as these may be readily derived from a decomposition of phosphatides, which exist in the fatty tissue between the nerve-axes, it is difficult to decide what part the fatty acids and neutral fats play as constituents of the real nerve-substance.

By allowing water to act on the contents of the medulla, round or oblong double-contoured drops or fibers, not unlike double-contoured nerves, are formed. These remarkable formations, which can also be seen in the medulla of the dead nerve, have been called "*myeline forms*," and they were formerly considered as produced from a special body, "myeline." Myeline forms may, however, be obtained from other bodies, such as impure protagon, lecithin, and impure cholesterin, and they depend upon a decomposition of the constituents of the medulla.

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[1] Koch, Amer. Journ. of Physiol., **11**; Kühne and Chittenden, Zeitschr. f Biologie, **26**; Baumstark, Zeitschr. f. physiol. Chem., **9**.

[2] v. Jaksch, Pflüger's Arch. **13**; Abderhalden and Weil, Zeitschr. f. physiol. Chem. **81** and **83**.

[3] Bioch. Zeitschr., **24**.

[4] Thudichum, Die chemische Konstitution des Gehirns des Menschen und der Tiere, Tübingen, 1901; S. Fränkel, and collaborators, Bioch. Zeitschr., **24** and **28**.

The *extractive bodies* seem to be almost the same as in the muscles. One finds *creatine*, which may, however, be absent (BAUMSTARK), *purine bases*, *inosite*, *choline*, *paralactic acid* (MORIYA), *phosphocarnic acid*, *uric acid*, and the diamine *neuridine*, $C_5H_{14}N_2$, discovered by BRIEGER[1] and which is most interesting because of its appearance in the putrefaction of animal tissues or in cultures of the typhoid bacillus. Among the enzymes we must mention *catalases*, *peroxidases*, *lipases* and *amylases* (WRÓBLEWSKI). According to the autolytic experiments of SIMON[2] a *proteolytic* enzyme, and an enzyme acting upon the organic phosphorized substance with the splitting off of phosphoric acid also occur. Under pathological conditions *leucine* and *urea* have been found in the brain. Urea is also a physiological constituent of the brain of cartilaginous fishes.

Several of the lipoids occurring in the brain have been discussed in previous chapters, and we will here only speak of the protagon and the cerebrosides.

Protagon. Under this name LIEBREICH described a crystalline, nitrogenous and phosphorized substance, which has been found in the brain of man, mammalia and also birds (ARGIRIS) but not in the brain of fishes (ARGIRIS). Its elementary composition, according to GAMGEE and BLANKENKORN, is C 66.39, H 10.69, N 2.39 and P 1.07 per cent. The results obtained by CRAMER correspond well with these figures and he found that protagon also contained sulphur which had previously been found by RUPPEL and by KOSSEL. Recently WILSON and CRAMER[3] have reported more recent analyses and they find for protagon, recrystallized 4–5 times, almost the same figures as GAMGEE and BLANKENHORN, namely, C 66.53, H 10.97, N 2.37, P 0.95 and S 0.73 per cent. They consider protagon as a unit substance.

GIES, POSNER and ROSENHEIM and TEBB[4] dispute the unit nature of protagon. They have found, on fractional precipitation or on recrystallization, that protagons can be obtained from the various solvents, having variable composition, especially different P and N contents. They

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[1] Brieger, Ueber Ptomaine, Berlin, 1885 and 1886.

[2] Wróblewski, Compt. Rend., **152**; Fr. Simon, Zeitschr. f. physiol. Chem., **72**.

[3] Liebreich, Annal. d. Chem. u. Pharm., **134**; Argiris, Zeitschr. f. physiol. Chem., **57**; Gamgee and Blankenhorn, *ibid.*, **3**; Kossel and Freytag, *ibid.*, **17**; Ruppel, Zeitschr. f. Biol., **31**; Cramer, Journ. of Physiol., **31**, with R. A. Wilson, Journ. of exp. Physiol., **1**, with Lockhead, Bioch. Journ., **2**; also Cramer, Quarterly Journ. of exp. Physiol. **3**, and Bioch. Handlexikon (Abderhalden) Bd. **3**, which contains the literature.

[4] Gies and Lesem, Amer. Journ. of Physiol., 8; Posner and Gies, Journ. of biol. Chem., **1**; Gies, *ibid.*, **3**; Rosenheim and Tebb, Journ. of Physiol., **36** and **37**, Quarterly Journ. of exp. Physiol., **2**, and Bioch. Zeitschr., **25**.

are, therefore, as are LESEM, THUDICHUM, WÖRNER and THIERFELDER,[1] and others, of the opinion that protagon does not exist as a chemical individual, but as a mixture of cerebrosides and phosphatides. It is not easy to come to any decision on this disputed question. On the one hand it must be recalled that several investigators call the impure mixture of brain lipoids, protagon, which they obtain from the solution in warm alcohol on cooking, and which is not purified, and this mixture is claimed to be identical, without sufficient basis, with the substance isolated and analyzed by GAMGEE and CRAMER. On the other hand it cannot be denied that certain investigations, especially those of ROSENHEIM and TEBB speak against the chemical individuality of protagon. These investigations do not exclude the possibility that protagon is a loose chemical combination between cerebroside and phosphatide, which like other readily dissociable combinations, exist only under certain conditions or in certain solvents. It is difficult to understand how a mixture of amorphous or only difficultly crystallizable bodies can be so easily crystallized and yield a product, which with proper care, can be recrystallized repeatedly without changing its composition, and physical properties. According to ROSENHEIM and TEBB if the proper quantity is used in solution, a crystalline product can be obtained from the decomposition products of protagon, which has the same specific rotation as protagon and can be repeatedly recrystallized without changing its composition or its optical activity.[2] A further study of these conditions would naturally be of great interest.

As we are not decided whether protagon is only a mixture or is a body contaminated with other substances, it is difficult to decide as to how far the so-called decomposition products exist as preformed constituents of the mixture or whether they are true decomposition products. On boiling with baryta-water protagon yields *cerebrosides* (see below) and the decomposition products of lecithin, namely, *fatty acids*, *glycerophosphoric acid*, and *choline*. KOSSEL and FREYTAG found three cerebrosides, namely, CEREBRIN, KERASIN (homocerebrin), and ENCEPHALIN. According to KOCH[3] the protagon molecule contains cerebroside, lecithin and sulphuric acid (in ester-like combination with the cerebroside) besides excess of cerebroside. Of interest is the finding of KITAGAWA and THIERFELDER[4] that protagon dissolved in methyl alcohol containing chloroform, deposits crusts of cerebron (not pure) after a time at

[1] Lesem, l. c.; Thudichum, l. c.; Wörner and Thierfelder, Zeitschr. f. physiol. Chem., **30**.

[2] Journ. of Physiol., **37**; Proc. physiol. Soc., January, 1908, p. 3.

[3] Zeitschr. f. physiol. Chem., **53**.

[4] Kitagawa and Thierfelder, *ibid.*, **49**; Rosenheim and Tebb, Journ. of Physiol., **37**, 341 and 348.

ordinary temperature, and that as shown by ROSENHEIM and TEBB, on dissolving in pyridine at 30° C. and heating or cooling the solution deposits a precipitate of a substance rich in phosphorus. Although we generally consider the phosphorized component of protagon as lecithin, still, according to ROSENHEIM and TEBB, it is probably a diamido-phosphatide, called *sphingomyelin* by THUDICHUM. On boiling protagon with dilute mineral acids it yields galactose, due to the decomposition of the cerebrosides.

Protagon appears, when dry, as a loose white powder. It dissolves in alcohol of 85 vols. per cent at 45° C., but separates on cooling as a snow-white, flaky precipitate, consisting of globules or groups of fine crystalline needles. On heating to 150° it becomes yellowish, softens at 180° and melts sharply at 200° forming a brown, oily liquid (CRAMER). It is difficultly soluble in cold alcohol or ether, but dissolves, at least when freshly precipitated, in ether on warming. It dissolves in methyl alcohol containing chloroform and, as above stated, separates cerebron. Protagon is soluble in pyridine at 30° C., yielding a clear solution, and this solution has a specific rotation $(\alpha)_D = +6.9$ to 7.7° according to the concentration of the solution (WILSON and CRAMER). On warming or cooling according to ROSENHEIM and TEBB, the rotation changes with the separation of sphingomyelin so that it first diminishes in rotation, then is zero, and then becomes strongly levorotatory until it reaches −242°, and finally, when nearly all the sphingomyelin has separated out it becomes constant at about −13.3°. The strong levorotation depends upon the accumulations of doubly refracting spheroid crystals of sphingomyelin. With little water protagon swells up and is partly decomposed. With more water it forms a jelly or pasty-like mass which, with the addition of considerable water, forms an opalescent liquid.

Protagon can be prepared in the following way: The finely ground brain-mass, as free as possible from blood and membrane, is dehydrated, which is best done by cold acetone or by grinding with burned plaster-of-paris or anhydrous sodium sulphate, and then extracted with ether. The mass is then extracted at 45° C. with 85 vol. per cent alcohol until the filtrate when cooled to 0° C. gives no more precipitate. All the precipitates obtained on cooling to 0° C. are extracted with ether and recrystallized from alcohol. Further details can be found in the cited works of CRAMER, WILSON, GIES, ROSENHEIM and TEBB.

Among the phosphatides occurring in the brain we must mention besides the lecithin and cephalin, the following substances.

Myelin, $C_{40}H_{75}NPO_{10}$, according to THUDICHUM, is not well known but is characterized by the fact that its alcoholic solution is not precipitated by $CdCl_2$ or $PtCl_4$. On the contrary an alcoholic solution of lead acetate gives a precipitate. The existence of a second monaminomonophosphatide, paramyelin, $C_{36}H_{72}NPO_9$, according to THUDICHUM, is very improbable.

Sphingomyelin, is a diaminomonophosphatide which THUDICHUM prepared from the brain and is the chief phosphatide obtainable from the impure protagon mixtures. ROSENHEIM and TEBB obtained it, as above mentioned, from the protagon. It has been given the formula $C_{42}H_{104}N_2PO_9+H_2O$. As cleavage products an alcohol, sphingol, neurin, cholin, according to ROSENHEIM and TEBB, the base sphingosin (see cerebron) and sphingostearic acid have been obtained. Sphingomyelin is soluble with difficulty in cold alcohol but readily soluble in hot alcohol and crystallizes therefrom in needles. It is insoluble in ether. In regard to the specific rotation see above in reference to protagon. *Amidomyelin* (Thudichum) is another diaminomonophosphatide of an unknown constitution and of an uncertain composition. Its existence is uncertain.

Sahidin was found by FRÄNKEL[1] in the brain, and is a triaminodiphosphatide, whose cadmium compound has the formula $C_{80}H_{167}N_3P_2O_{12}.3CdCl_2$. It is a crystalline powder which is insoluble in water, cold ethyl or methyl alcohol and in ether. It is soluble with difficulty in warm alcohol but readily soluble in chloroform and hot benzene. It yields saturated and unsaturated fatty acids, choline and glycerophosphoric acid.

Leucopoliin is an unsaturated phosphatide found by FRÄNKEL and ELIAS[2] in the brain and which is a decaminodiphosphatide or a pentaminomonophosphatide. It crystallizes from boiling alcohol on cooling. It does not contain any methylated base but does contain a carbohydrate group.

Sulphatide is the name given by KOCH[3] to a sulphurized and phosphorized product obtained from the human brain which separates from warm pyridine on cooling as a crystalline, granular mass. It contains phosphatide, sulphuric acid and cerebroside and is claimed to be phosphatidesulphuric acid cerebroside.

Cerebrosides.

On decomposing protagon (or the protagons), or the brain substance by the gentle action of alkalies we obtain, as cleavage products, as above stated, one or more bodies which THUDICHUM has embraced under the name *cerebrosides*. The cerebrosides are nitrogenous substances free from phosphorus, which yield galactose on boiling with dilute mineral acids. With concentrated sulphuric acid they first give a yellow and then a purple-red coloration. With sulphuric acid and cane-sugar they give a purple coloration directly. The cerebrosides isolated from the brain are cerebrin, homocerebrin, phrenosin, kerasin, encephalin, and cerebron, but it must be remarked that there is no doubt that sometimes the same body of varying purity has received different names. According to LEVENE and JACOBS[4] it must be admitted that the cerebrosides are mixtures of stereoisomeric substances.

Cerebrin. Under this name W. MÜLLER[5] first described a nitrogenous substance, free from phosphorus, which he obtained by extracting, with boiling alcohol, a brain-mass which had been previously boiled with

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[1] Bioch. Zeitschr. **24.**
[2] Fränkel and Elias, Bioch. Zeitschr. **28.**
[3] Zeitschr. f. physiol. Chem. **70.**
[4] Journ. of biol. Chem. **12.**
[5] Annal. d. Chem. u. Pharm., **105.**

baryta-water. Following a method essentially the same, but differing slightly, GEOGHEGAN prepared, from the brain, a cerebrin with the same properties as MÜLLER'S, but containing less nitrogen. According to PARCUS[1] the cerebrin isolated by GEOGHEGAN, as well as by MÜLLER, consists of a mixture of three bodies, "cerebrin," "homocerebrin," and "encephalin." KOSSEL and FREYTAG isolated two cerebrosides from protagon which were identical with the cerebrin and homocerebrin of PARCUS. According to these investigators, the two bodies phrenosin and kerasin, as described by THUDICHUM, seem to be identical with cerebrin and homocerebrin.

Cerebrin, according to PARCUS, has the following composition: C 69.08, H 11.47, N 2.13, O 17.32 per cent, which corresponds with the analyses made by KOSSEL and FREYTAG. No formula has been given to this body. In the dry state it forms a pure white, odorless, and tasteless powder. On heating it melts, decomposes gradually, smells like burned fat, and burns with a luminous flame. Melting-point is 170–176° C. It is insoluble in water, dilute alkalies, or baryta-water; also in cold alcohol and in cold or hot ether. On the contrary, it is soluble in boiling alcohol and separates as a flaky precipitate on cooling, and this is found to consist of a mass of globules or grains on microscopical examination. Cerebrin forms a compound with baryta, which is insoluble in water and is decomposed by the action of carbon dioxide. The variety of sugar split off on boiling with mineral acids—the so-called brain-sugar—is, as THIERFELDER[2] first showed, galactose. On cleavage with nitric acid fatty acids (stearic acid) were obtained.

Kerasin (THUDICHUM), or *homocerebrin* (PARCUS), has the following composition: C 70.06, H 11.60, N 2.23, and O 16.11 per cent. **Encephalin** has the composition C 68.40, H 11.60, N 3.09, and O 16.91 per cent. Both bodies remain in the mother-liquor after the impure cerebrin has precipitated from the warm alcohol. These bodies have the tendency of separating as gelatinous masses. Kerasin is similar to cerebrin, but dissolves more easily in warm alcohol and also in warm ether. It may be obtained as extremely fine needles. Encephalin is, PARCUS thinks, a transformation product of cerebrin. In the perfectly pure state it crystallizes in small lamellæ. It swells in warm water into a pasty mass.

As the purity and the chemical individuality of the above-mentioned bodies is questionable, it is perhaps sufficient in regard to their preparation to simply call attention to the cited works of MÜLLER, GEOGHEGAN, KOSSEL and FREYTAG. All these methods split with barium hydroxide and purify the cerebroside by solution in hot alcohol and a precipitation by cooling.

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[1] Geoghegan, Zeitschr. f. physiol. Chem., **3**; Parcus, Ueber einige neue Gehirnstoffe, Inaug.-Diss. Leipzig, 1881.

[2] Zeitschr. f. physiol. Chem., **14**.

Whether the above-described cerebrosides are chemical individuals or mixtures, i. e., impure substances, is still undecided. The purest cerebroside thus far investigated is undoubtedly THIERFELDER'S cerebron, and there is hardly any doubt that the above-mentioned cerebrosides consist essentially of this body.

Cerebron. This cerebrin, isolated by THIERFELDER and WÖRNER and then especially studied by THIERFELDER, was first isolated by GAMGEE and called *pseudocerebrin* by him. THUDICHUM'S *phrenosin* is, according to GIES,[1] identical with cerebron. Cerebron can be prepared directly from the brain without saponification with baryta, by treatment with alcohol containing benzene or chloroform at a temperature of 50°, and hence it is considered as existing preformed in the brain. According to THIERFELDER, cerebron has the formula $C_{42}H_{93}NO_9$; it melts at 212°, dissolves in warm alcohol, and separates out on cooling. From proper solvents (acetone or methyl alcohol containing chloroform) it may be separated as small needles or plates. If cerebron is suspended in 85-per cent alcohol at a temperature of 50° C. it balls together in amorphous masses, and from these needle- and leaf-shaped crystals gradually form. It is dextrorotatory, and in about a 5-per cent solution in methyl alcohol (containing 75 per cent chloroform) is $(\alpha)_D = +7.6°$ (KITAGAWA and THIERFELDER). According to THIERFELDER it yields as cleavage products, *galactose*, *cerebronic acid* (THUDICHUM "*neurostearic acid*") and *sphingosin* which is in part obtained as such and part as dimethylsphingosin. The base sphingosin, $C_{17}H_{35}NO_2$, discovered by THUDICHUM, is, according to THIERFELDER and to LEVENE and JACOBS,[2] an unsaturated, diatomic, monoamino-alcohol which is readily soluble in alcohol, ether, acetone and petroleum ether but insoluble in water, nas an alkaline reaction and has not been obtained in a crystalline state. The sulphate of dimethylsphingosin crystallizes, on the contrary, from alcohol. Cerebronic acid is an oxyacid with the formula $C_{25}H_{50}O_3$, which is crystalline and which gives a crystalline methyl ester which melts at 65° C. It has been obtained by LEVENE and JACOBS[3] in part in a dextrorotatory and in part as an inactive form. The first melts at 106–108° and the other at 82–85° C.

Cerebron can best be prepared, according to THIERFELDER and KITAGAWA, by decomposing the protagon in methyl alcohol containing

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[1] Thierfelder and Wörner, Zeitschr. f. physiol. Chem., **30**; Thierfelder, *ibid.*, **42**, **44**, **46**, with Kitagawa, *ibid.*, **49**; with H. Loening, *ibid.*, **68**, **74**, **77**; Gamgee, Textbook of Physiol. Chem., London, 1880; Thudichum, l. c.; Gies, Journ. of Biol. Chem., **1** and **2**.

[2] Thierfelder and O. Riesser and K. Thomas, Zeitschr. f. physiol. Chem., **77**; Levene and Jacobs, Journ. of biol. Chem. **11**.

[3] Journ. of biol. Chem., **12**.

chloroform (see page 607), and purifying the separated cerebron from contaminating phosphatides by precipitating these with an ammoniacal solution of zinc hydroxide in methyl alcohol, and recrystallizing the cerebron from methyl alcohol containing chloroform. THIERFELDER and LOENING have devised another method of purification and at the same time they have suggested another method for preparing cerebron. This method is based upon the resistance of the cerebrosides to baryta and their solubility in hot acetone. It consists in boiling the impure protagon mixture with baryta-water and boiling the insoluble residue with acetone.

Neuridine, $C_5H_{14}N_2$, is a non-poisonous diamine discovered by BRIEGER, and obtained by him in the putrefaction of meat and gelatin, and from cultures of the typhoid bacillus. It also occurs under physiological conditions in the brain, and as traces in the yolk of the egg.

Neuridine dissolves in water and yields on boiling with alkalies a mixture of dimethylamine and trimethylamine. It dissolves with difficulty in amyl alcohol. It is insoluble in ether or absolute alcohol. In the free state, neuridine has a peculiar odor, suggesting semen. With hydrochloric acid it gives a compound crystallizing in long needles. With platinic chloride or gold chloride it gives crystallizable double compounds which are valuable in its preparation and detection.

The so-called CORPUSCULA AMYLACEA, which occur on the upper surface of the brain and in the pituitary gland, are colored more or less pure violet by iodine and more blue by sulphuric acid and iodine. They perhaps consist of the same substance as certain prostatic calculi, but they have not been closely investigated.

Quantitative Composition of the Brain. The quantity of water is greater in the gray than in the white substance, and greater in new-born or young individuals than in adults. The brain of the fœtus contains 879–926 p. m. water. The observations of WEISBACH[1] show that the quantity of water in the several parts of the brain (and in the medulla) varies at different ages. The following figures are in 1000 parts—*A* for men and *B* for women:

	20–30 years.		30–50 years.		50–70 years.		70–94 years.	
	A.	*B.*	*A.*	*B.*	*A.*	*B.*	*A.*	*B.*
White brain-substance..	695.6	682.9	683.1	703.1	701.9	689.6	726.1	722.0
Gray "	833.6	826.2	836.1	830.6	838.0	838.4	847.8	839.5
Gyri.....	784.7	792.0	795.9	772.9	796.1	796.9	802.3	801.7
Cerebellum.....	788.3	794.9	778.7	789.0	787.9	784.5	803.4	797.9
Pons Varolii.....	734.6	740.3	725.5	722.0	720.1	714.0	727.4	724.4
Medulla oblongata.....	744.3	740.7	732.5	729.8	722.4	730.6	736.2	733.7

The recent investigations of K. LINNERT[2] correspond to the above in that the pons and the medulla were found to be next to the white substance, the poorest in water, of the human brain.

Quantitative analyses of human brains at different ages, namely 6 weeks, 2 and 19 years, have been made by KOCH and MANN.[3] These analyses show that with increasing age the water, proteins, extractives

[1] Cited from K. B. Hoffmann's Lehrbuch d. Zioch., Wien, 1877, p. 121.

[2] Wien. klin. Wochenschr., **23**.

[3] Journ. of Physiol., **36**, Proc. physiol. Soc., 1907.

and salts diminish relatively, while the phosphatides, cerebrosides and especially cholesterin strikingly increase. The sulphur of the lipoids increased to the second year, but then existed in the same amounts as at nineteen years.

BAUMSTARK claims to have found that a part of the cholesterin in the brain occurs in a combined state, perhaps as ester; this view has been found to be incorrect by the recent investigations of BÜNZ. He obtained from the brain neither esters of cholesterin with higher fatty acids nor other compounds of cholesterin which split on saponification. TEBB [1] has also found only free cholesterin.

According to FRÄNKEL,[2] who has fractionally extracted the human brain with various solvents, found 230 p. m. solids in the brain and this consisted of $\frac{2}{3}$ lipoids and $\frac{1}{3}$ proteins. Of the lipoids about 17 per cent was cholesterin, 34.482 per cent saturated and 48.293 per cent unsaturated compounds. The amount of cholesterin in the different parts of the brain was as follows, according to FRÄNKEL, KIRSCHBAUM and LINNERT. In the cortex 11.5 p. m., in the white substance 24.7 p. m., in the cerebellum 13.1 p. m., and in the bridge and medulla 40.3 p. m., all calculated in the moist substance.

The analysis of the brain of an epileptic made by KOCH [3] is of very great interest. As the protagon is considered by KOCH as a mixture, no results for the quantity of protagon are given. As no accurate methods for the estimation of the little known bodies cephalin, myelin, phrenosin and kerasin are available, the figures given for these are of little value. The following results are calculated to 1000 parts:

	Corpus Callosum		Cortex (prefrontal).	
Water	679.7		841.3	
Protein	32.0		50.0	
Nucleoproteins	37.0		30.0	
Neurokeratin	27.0	(CHITTENDEN)	4.0	(CHITTENDEN)
Extractives (water-soluble)	15.1		15.8	
Lecithins	51.9		31.4	
Cephalin and myelin	34.9		7.4	
Phrenosin and kerasin	45.7		15.5	
Cholesterin	48.6		7.0	
Sulphurized substance	14.0		14.5	
Mineral bodies	8.2		8.7	

PIGHINI and CARBONE found that the brains of paralytics were richer in water, considerably richer in cholesterin, but poorer in cephalin than healthy brains. This last corresponds, to the observations of KOCH and MANN [4] that the quantity of lipoid phosphorus was diminished in paralytics.

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[1] Baumstark, Zeitschr. f. physiol. Chem. **9**; R. Bünz *ibid.*, **46**; Tebb, Journ. of Physiol., **34**.

[2] Bioch. Zeitschr., **19**, with Kirschbaum and Linnert, *ibid.*, **46**.

[3] Amer. Journ. of Physiol., **11**.

[4] Pighini and Carbone, Bioch. Zeitschr., **46**; Koch and Mann, Arch. of Neurol. and Psychol., 1910.

According to Fr. Falk[1] the cerebrosides occur in the medullary nerve fibers as well as in the nerves without medullas. These latter yielded much less substance on extraction than the medullary, namely, 11.51 per cent extract as compared to 46.59 per cent. The extract of the first was poorer in cerebrosides, but richer in cholesterin, cephalin and lecithin, as shown by the following figures.

	Non-medullary fibers in p. m. of the total extract	Medullary fibers in p. m. of the total extract
Cholesterin	470	250
Cephalin	237	124
Cerebrosides	60	182
Lecithins	98	29

S. Fränkel and L. Dimitz[2] find that the spinal marrow contains on an average 740 p. m. water, 180 p. m. lipoids and 80 p. m. protein. The quantity of cholesterin (in the fresh, spinal marrow containing water) is 40 p. m., the unsaturated phosphatide 120 p. m., and the saturated 15 p. m. The spinal marrow is the richest part of the nervous system in unsaturated phosphatides and it contains abundance of cephalin.

According to Noll the white substance of the spinal marrow is somewhat richer in protagon than the brain, and in nerve degeneration the quantity of protagon diminishes. The method used by him would not allow of an exact determination of the disputed substance protagon. Mott and Halliburton[3] have also shown that in degenerative diseases of the nervous system, the quantity of substances containing phosphorus diminishes, and that in these cases, especially in general paralysis, choline passes into the cerebrospinal fluid and the blood. In degenerated nerves, the quantity of water increases, and the phosphorus decreases. On comparative investigations of the central nervous system of normal persons, and those afflicted with dementia præcox (5 cases), Koch[4] found that the variation from the normal composition was not great enough nor so constant that positive conclusions could be drawn therefrom.

The quantity of neurokeratin in the nerves and the different parts of the brain has been carefully determined by Kühne and Chittenden.[5] They found 3.16 p. m. in the plexus brachialis, 3.12 p. m. in the cortex of the cerebellum, 22.434 p. m. in the white substance of the cerebrum, 25.72–29.02 p. m. in the white substance of the corpus callosum, and 3.27 p. m. in the gray substance of the cortex of the cerebrum (when

[1] Bioch. Zeitschr., **18**.

[2] *Ibid.*, **28**.

[3] Noll, Zeitschr. f. physiol. Chem., **27**; Mott and Halliburton, Philos. Transactions Ser. B., **191** (1899), and **194** (1901).

[4] Arch. of Neurology, **3**.

[5] Zeitschr. f. Biologie, **26**.

free as possible from white substance). The white is decidedly richer in neurokeratin than the peripheral nerves or the gray substance. According to GRIFFITHS,[1] *neurochitin* replaces neurokeratin in insects and crustacea, the quantity of the first being 10.6–12 p. m.

The quantity of mineral constituents in the brain amounts to 2.95–7.08 p. m. according to GEOGHEGAN. He found in 1000 parts of the fresh, moist brain 0.43–1.32 Cl, 0.956–2.016 PO_4, 0.244–0.796 CO_3, 0.102–0.220 SO_4, 0.01–0.098 $Fe_2(PO_4)_2$, 0.005–0.022 Ca, 0.016–0.072 Mg, 0.58–1.778 K, and 0.450–1.114 Na. The gray substance yields an alkaline ash, the white an acid ash. MAGNUS-LEVY [2] found in fresh brain substance 1.305 p. m. Cl, 0.166 p. m. Ca, 0.139 p. m. Mg, and 0.083 p. m. Fe.

Appendix.

THE TISSUES AND FLUIDS OF THE EYE.

The retina contains in all 865–899.9 p. m. water, 57.1–84.5 p. m. protein bodies—myosin, albumin, and mucin (?), 9.5–28.9 p. m. lecithin, and 8.2–11.2 p. m. salts (HOPPE-SEYLER and CAHN [3]). The mineral bodies consist of 422 p. m. Na_2HPO_4 and 352 p. m. NaCl. The retina contains, according to BARBIERI,[4] also cholesterin but no cerebrosides and in fact none of the specific constituents of the brain substance.

Those bodies which form the different segments of the rods and cones have not been closely studied, and the greatest interest is therefore connected with the coloring-matters of the retina.

Visual purple, also called *rhodopsin*, *erythropsin*, or VISUAL RED, is the pigment of the rods. BOLL,[5] in 1876, observed that the layer of rods in the retina during life had a purplish-red color which was bleached by the action of light. KÜHNE [6] later showed that this red color might remain for a long time after the death of the animal if the eye was protected from daylight or investigated by a sodium light. Under these conditions it was also possible to isolate and closely study this substance.

Visual red (BOLL) or visual purple (KÜHNE) has become known mainly by the investigations of KÜHNE. The pigment occurs mainly in the rods and only in their outer parts. In animals whose retina has no rods the visual purple is

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[1] Compt. Rend., 115.

[2] Geoghegan, Zeitschr. f. physiol Chem.. 1; Magnus-Levy, Bioch. Zeitschr. 24.

[3] Zeitschr. f. physiol. Chem., 5.

[4] Compt. Rend., 154.

[5] Monatsber. d. Kgl. Preuss. Akad., 12. Nov., 1876.

[6] The investigations of Kühne and his pupils, Ewald and Ayres, on the visual purple will be found in Untersuchungen aus dem physiol. Institut der Universität Heidelberg, 1 and 2, and in Zeitschr. f. Biologie, 32.

absent, and is also necessarily absent in the macula lutea. In a variety of bat (Rhinolophus hipposideros), in hens, pigeons and new-born rabbits, no visual purple has been found in the rods.

A solution of visual purple in water which contains 2–5 per cent crystallized bile, which is the best solvent for it, is purple-red in color, quite clear, and not fluorescent. On evaporating this solution *in vacuo* we obtain a residue similar to ammonium carminate which contains violet or black grains. If the above solution is dialyzed with water, the bile diffuses and the visual purple separates as a violet mass. Under all circumstances, even when still in the retina, the visual purple is quickly bleached by direct sunlight, and with diffused light with a rapidity corresponding to the intensity of the light. It passes from red and orange to yellow. Red light bleaches the visual purple slowly; the ultra-red light does not bleach it at all. A solution of visual purple shows no special absorption bands, but only a general absorption which extends from the red side, beginning at *D* and extending to the *G* line. The strongest absorption is found at *E*.

KOETTGEN and ABELSDORF [1] have shown that there are, in accordance with KÜHNE'S views, two varieties of visual purple, the one occurring in mammals, birds, and amphibians, and the other, which is more violet-red, in fishes. The first has its maximum absorption in the green and the other in the yellowish-green.

Visual purple when heated to 52–53° C. is destroyed after several hours, and almost instantly when heated to 76° C. It is also destroyed by alkalies, acids, alcohol, ether, and chloroform. On the contrary, it resists the action of ammonia or alum solution.

As the visual purple is easily destroyed by light, it must therefore also be regenerated during life. KÜHNE has also found that the retina of the eye of the frog becomes bleached when exposed for a long time to strong sunlight, and that its color gradually returns when the animal is placed in the dark. This regeneration of the visual purple is a function of the living cells in the layer of the pigment epithelium of the retina. This may be inferred from the fact that a detached piece of the retina which has been bleached by light may have its visual purple restored if it is carefully laid on the choroid having layers of the pigment-epithelium attached. The regeneration has, it seems, nothing to do with the dark pigment, the melanin or fuscin, in the epithelium cells. A partial regeneration seems, according to KÜHNE, to be possible in the retina which has been completely removed. On account of this property of the visual purple of being bleached by light during life we may, as KÜHNE has shown, under special conditions and by observing special precautions, obtain after death, by the action of intense light or more continuous light, the picture of bright objects, such as windows and the like—so-called optograms.

The physiological importance of visual purple is unknown. It follows that the visual purple is not essential to sight, since it is absent in certain animals and also in the cones.

[1] Centralbl. f. Physiol., 9; also Maly's Jahresber., 25, 351.

Visual purple must always be prepared exclusively in a sodium light. It is extracted from the net membrane by means of a watery solution of crystallized bile. The filtered solution is evaporated *in vacuo* or dialyzed until the visual purple is separated. To prepare a visual-purple solution perfectly free from hæmoglobin, the solution of visual purple in cholates is precipitated by saturating with magnesium sulphate, washing the precipitate with a saturated solution of magnesium sulphate, and then dissolving in water by the aid of the cholates simultaneously precipitated.[1]

The Pigments of the Cones. In the inner segments of the cones of birds, reptiles, and fishes a small fat-globule of varying color is found. KÜHNE[2] has isolated from this fat a green, a yellow, and a red pigment called respectively *chlorophan*, *xanthophan*, and *rhodophan*.

The dark pigment of the epithelium-cells of the net membrane, which was formerly called *melanin*, but has since been named *fuscin* by KÜHNE and MAYS,[3] contains iron, dissolves in concentrated caustic alkalies or concentrated sulphuric acid on warming, but, like the melanins in general, has been little studied. The pigment occurring in the pigment-cells of the choroid will be discussed with the melanins in Chapter XV.

The **vitreous humor** is often considered as a variety of gelatinous tissue. The membrane consists, according to C. MÖRNER, of a gelatin-forming substance. The fluid contains a little proteid and a mucoid, *hyalomucoid*, which was first shown by MÖRNER, and which is precipitated by acetic acid. This contains 12.27 per cent N, and 1.19 per cent S. Among the extractives we find a little *urea*—according to PICARD 5 p. m., according to RÄHLMANN 0.64 p. m. PAUTZ[4] found besides some urea, *paralactic acid*, and, in confirmation of the claims of CHABBAS, JESNER, and KUHN, also *glucose* in the vitreous humor of oxen. The reaction of the vitreous humor is alkaline, and the quantity of solids amounts to about 9–11 p. m. The quantity of mineral bodies is about 6–9 p. m., and the proteins 0.7 p. m. In regard to the aqueous humor see page 361.

The Crystalline Lens. That substance which forms the capsule of the lens has been investigated by C. MÖRNER. It belongs, according to him, to a special group of proteins, called *membranins*. The membranin bodies are insoluble at the ordinary temperature in water, salt solutions, dilute acids, and alkalies, and, like the mucins, yield a reducing substance on boiling with dilute mineral acids. They contain lead-blackening sulphur. The membranins are colored a very beautiful red by MILLON'S reagent, but give no characteristic reaction with concentrated hydrochloric acid or ADAMKIEWICZ'S reagent. They are dissolved with

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[1] Kühne, Zeitschr. f. Biologie, **32**.

[2] Kühne, Die nichtbeständigen Farben der Netzhaut, Untersuch. aus dem physiol. Institut Heidelberg, **1**, 341.

[3] Kühne, *ibid.*, **2**, 324.

[4] Mörner, Zeitschr. f. physiol. Chem., **18**; Picard, cited from Gamgee, Physiol. Chem., **1**, 454; Rählmann, Maly's Jahresber., **6**; Pautz, Zeitschr. f. Biologie, **31**. A complete review of the literature will also be found here.

great difficulty by pepsin-hydrochloric acid or trypsin solution, but are soluble in dilute acids and alkalies in the warmth. Membranin of the capsule of the lens contains 14.10 per cent N and 0.83 per cent S, and is a little less soluble than that from DESCEMET'S membrane.

The principal mass of the solids of the crystalline lens consists of proteins, whose nature has been investigated by C. MÖRNER.[1] Some of these proteins dissolve in dilute salt solution, while others remain insoluble in this solvent.

The Insoluble Protein. The lens fibers consist of a protein substance which is insoluble in water and in salt solution and to which MÖRNER has given the name *albumoid.* It dissolves readily in very dilute acids or alkalies. Its solution in caustic potash of 0.1 per cent is very similar to an alkali-albuminate solution, but coagulates at about 50° C. on nearly complete neutralization and the addition of 8 per cent NaCl. Albumoid has the following composition: C 53.12, H 6.8, N 16.62, and S 0.79 per cent. The lens fibers themselves contain 16.61 per cent N and 0.77 per cent S. The inner parts of the lens are considerably richer in albumoid than the outer. The quantity of albumoid in the entire lens amounts on an average to about 48 per cent of the total weight of the proteins of the lens.

The Soluble Protein consists, exclusive of a very small quantity of *albumin*, of two globulins, α- and *β-crystallin.* These two globulins differ from each other in this manner: α-crystallin contains 16.68 per cent N and 0.56 per cent S; β-crystallin, on the contrary, 17.04 per cent N and 1.27 per cent S. The first coagulates at about 72° C. and the other at 63° C. Besides this, β-crystallin is precipitated from a salt-free solution with greater difficulty and less completely by acetic acid or carbon dioxide. These globulins are not precipitated by an excess of NaCl at either the ordinary temperature or 30° C. Magnesium or sodium sulphate in substance precipitates both globulins, on the contrary, at 30° C. These two globulins are not equally divided in the mass of the lens. The quantity of α-crystallin diminishes in the lens from without inward; β-crystallin, on the contrary, from within outward.

A. JESS[2] has found that the different proteins of the crystalline lens behave differently with ARNOLD'S protein reaction with sodium nitroprusside (page 100). The albumoid gives negative results with this reagent. The α-crystallin gives it faintly, while the β-crystallin gives a strong reaction. The absence of this reaction, as observed by WEISS in senile cataract, is connected with the fact as JESS has shown by his investigations on the senile cataract in oxen, that the crystallin con-

[1] Zeitschr. f. physiol. Chem., 18. This contains also the pertinent literature.
[2] Zeitschr. f. Biol., 61.

taining cysteine, disappears in part from the lens and is partly transformed into albumoid. The relation between albumoid and crystallins is changed with increasing age, so that the albumoid increases. In normal lens the relation of the crystallins to the albumoid changes correspondingly from 82:18 in youth to 41:59 in old age; in senile cataract the relation can be changed to 25:75. The amount of fat, cholesterin and lecithin is on the contrary not changed.

The average results of four analyses made by LAPTSCHINSKY[1] of the lens of oxen are here given, calculated in parts per 1000:

Proteins	349.3
Lecithin	2.3
Cholesterin	2.2
Fat	2.9
Soluble salts	5.3
Insoluble salts	2.4

In cataract the amount of proteins is diminished and the amount of cholesterin increased. This statement requires further substantiation.[2]

The quantity of the different proteins in the fresh moist lens of oxen is, as follows, according to MÖRNER:

Albumoid (lens fibers)	170 p. m.
β-Crystallin	110 "
α-Crystallin	68 "
Albumin	2 "

The **corneal tissue** has been previously considered (page 550). The **sclerotic** has not been closely investigated, and the **choroid coat** is principally of interest because of the coloring-matter (melanin) it contains (see Chapter XV).

Tears consist of a water-clear, alkaline fluid of a salty taste. According to the analyses of LERCH[3] they contain 982 p. m. water, 18 p. m. solids with 5 p. m. albumin and 13 p. m. NaCl.

THE FLUIDS OF THE INNER EAR.

The **perilymph** and **endolymph** are alkaline fluids, which, besides salts, contain—in the same amounts as in transudates—traces of *protein*, and in certain animals (codfish) also *mucin*. The quantity of mucin is greater in the perilymph than in the endolymph.

Otoliths contain 745–795 p. m. inorganic substance, which consists chiefly of crystallized calcium carbonate. The organic substance is very similar to mucin.

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[1] Pflüger's Arch., 18.

[2] See Gross, Arch. f. Augenheilk., 55 and 58.

[3] Cited from v. Gorup-Besanez, Lehrbuch d. physiol. Chem., 4 Aufl., 401.

CHAPTER XII.

ORGANS OF GENERATION.

(a) Male Generative Secretions.

The **testes** have been little investigated chemically. In the testes of animals we find protein bodies of different kinds—*seralbumin*, *alkali albuminate* (?), and an albuminous body related to ROVIDA'S *hyaline substance;* also *leucine*, *tyrosine*, *creatine*, *purine bases*, *cholesterin*, *lecithin*, *inosite*, and *fat*. In regard to the occurrence of glycogen the reports are conflicting. DARESTE [1] found, in the testes of birds, starch-like granules, which were colored blue with difficulty by iodine.

In the autolysis of the testes LEVENE [2] found tyrosine, alanine, leucine, aminovaleric acid, aminobutyric acid, α-proline, phenylalanine, aspartic acid, glutamic acid, and hypoxanthine. Pyrimidine and hexone bases could not be detected.

The **semen** as ejected is a white or whitish-yellow, viscous, sticky fluid of a milky appearance, with whitish, non-transparent lumps. The milky appearance is due to spermatozoa. Semen is heavier than water, contains proteins, has a neutral or faintly alkaline reaction and a peculiar specific odor. Soon after ejection semen becomes gelatinous, as if it were coagulated, but afterward becomes more fluid. When diluted with water white flakes or shreds separate (HENLE'S *fibrin*). According to the analyses of SLOWTZOFF,[3] human semen contains on an average 96.8 p. m. solids with 9 p. m. inorganic and 87.8 p. m. organic substance. The amount of protein substances was, on an average, 22.6 p. m. and 1.69 p. m. of bodies soluble in ether. The protein substances consist of *nucleoproteins*, traces of *mucin*, *albumin*, and a substance similar to *proteose* (found earlier by POSNER). According to CAVAZZANI semen contains relatively considerable *nucleon*, more than any organ. v. HOFFMANN [4]

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[1] Compt. Rend., **74**.

[2] Amer. Journ. of Physiol., **11**.

[3] Zeitschr. f. physiol. Chem., **35**.

[4] Posner, Berl. klin. Wochenschr., 1888, No. 21, and Centralbl. f. d. med. Wissensch., 1890; Cavazzani, Biochem. Centralbl., **1**, 502, and Centralbl. f. Physiol., **19**; v. Hoffmann, cited in Bioch. Centralbl., **9**, 206.

has found a protamine in human semen which yielded arginine and perhaps also lysine on cleavage. The mineral bodies consist mainly of calcium phosphate and considerable NaCl. Potassium occurs only in smaller amounts.

The semen in the vas deferens differs chiefly from the ejected semen in that it is without the peculiar odor. This last depends on the admixture with the secretion of the prostate. This secretion, according to IVERSEN, has a milky appearance and ordinarily an alkaline reaction, very rarely a neutral one, and contains small amounts of proteins, especially *nucleoproteins*, besides a substance similar to *fibrinogen* and to *mucin* (STERN [1]), and mineral bodies, especially NaCl. Besides this it contains an enzyme *vesiculase* (see below), *lecithin*, *choline* (STERN), and a crystalline combination of phosphoric acid with a base, C_2H_5N. This combination has been called BÖTTCHER'S *spermine crystals*, and it is claimed that the specific odor of the semen is due to a partial decomposition of these crystals.

The crystals which appear on slowly evaporating the semen, and which are also observed in anatomical preparations kept in alcohol, are not identical with the CHARCOT-LEYDEN crystals found in the blood and in the lymphatic glands in leucæmia (TH. COHN, B. LEWY [2]). They are, according to SCHREINER,[3] as above stated, a combination of phosphoric acid with a base, *spermine*, C_2H_5N, which he discovered.

Spermine. Opinions in regard to the nature of this base are not unanimous According to the investigations of LADENBURG and ABEL, it is not improbable that spermine is identical with ethylenimine; but this identity is disputed by MAJERT and A. SCHMIDT, and also by POEHL. The compound of spermine with phosphoric acid—BÖTTCHER'S spermine crystals—is insoluble in alcohol, ether, and chloroform, soluble with difficulty in cold water, but more readily in hot water, and easily soluble in dilute acids or alkalies, also alkali carbonates and ammonia. The base is precipitated by tannic acid, mercuric chloride, gold chloride, platinic chloride, potassium-bismuth iodide, and phosphotungstic acid. Spermine has a tonic action, and, according to POEHL,[4] it has a marked action on the oxidation processes of the animal body.

On the addition of a solution of potassium iodide and iodine to spermatozoa, characteristic dark-brown or bluish-black crystals are obtained—FLORENCE'S sperm reaction, which is considered by many as a reaction for spermine. According to BOCARIUS,[5] this reaction is due to choline.

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[1] Iversen, Nord. med. Ark., **6**; also Maly's Jahresber., **4**, 358; Stern, Biochem. Centralbl., **1**, 748.

[2] Th. Cohn, Centralbl. f. allg. Path. u. path. Anat., **10** (1899), and Zeitschr. f. Urolog., **1908**; B. Lewy, Centralbl. f. d. med. Wissensch., 1899, 479.

[3] Annal. d. Chem. u. Pharm., **194**.

[4] Ladenburg and Abel, Ber. d. deutsch. chem. Gesellsch., **21**; Majert and A. Schmidt, *ibid.*, **24**; Poehl, Compt. Rend., **115**, Berlin, klin. Wochenschr., 1891 and **1893**, Deutsch. med. Wochenschr., 1892 and 1895, and Zeitschr. f. klin. Med., 1894.

[5] In regard to Florence's sperm reaction, see Posner, Berl. klin. Wochenschr., **1897**, and Richter, Wien. klin. Wochenschr., 1897; Bocarius, Zeitschr. f. physiol. Chem., **24**.

CAMUS and GLEY [1] have found that the prostate fluid in certain rodents has the property of coagulating the contents of the seminal vesicles. This property is due to a special ferment substance (*vesiculase*) of the prostate fluid.

The spermatozoa show a great resistance to chemical reagents in general. They do not dissolve completely in concentrated sulphuric acid, nitric acid, acetic acid, or in boiling-hot soda solutions. They are soluble in a boiling-hot caustic-potash solution. They resist putrefaction, and after drying they may be obtained again in their original form by moistening them with a 1-per cent common-salt solution. By careful heating and burning to an ash the shape of the spermatozoa may be seen in the ash. The quantity of ash is about 50 p. m. and consists mainly (three-quarters) of potassium phosphate.

The spermatozoa show well-known movements, but the cause of this is not known. These movements may continue for a very long time, as under some conditions they may be observed for several days in the body after death, and in the secretion of the uterus longer than a week. Acid liquids stop these movements immediately; they are also destroyed by strong alkalies, especially ammoniacal liquids, also by distilled water, alcohol, ether, etc. The movements continue for a longer time in faintly alkaline liquids, especially in alkaline animal secretions, and also in properly diluted neutral salt solutions.[2]

Spermatozoa are nucleus formations and hence are rich in nucleic acid, which exists in the heads. The tails contain protein, and are besides this rich in lecithin, cholesterin, and fat, which bodies occur only to a small extent (if at all) in the heads. The tails seem by their composition to be closely allied to the non-medullated nerves or the axis-cylinders. In the various kinds of animals investigated, the head contains nucleic acid, which in fishes is partly combined with protamines and partly with histones. In other animals, such as the bull and boar, protein-like substances occur with the nucleic acid, but no protamine.

Our knowledge of the chemical composition of spermatozoa has been greatly enhanced by the important investigations of MIESCHER [3] on salmon milt. The intermediate fluid of the spermatozoa of Rhine salmon is a dilute salt solution containing 1.3–1.9 p. m. organic and 6.5–7.6 p. m. inorganic bodies. The last consist principally of sodium chloride and carbonate, besides some potassium chloride and sulphate. The fluid contains only traces of protein, but no peptone. The tails consist of 419 p. m. protein, 318.3 p. m. lecithin, and 262.7 p. m. cholesterin and

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[1] Compt. rend. de soc. biolog., 48, 49.

[2] See G. Günther, Pflüger's Arch., 118.

[3] See Miescher, "Die histochemischen und physiologischen Arbeiten von Friedrich Miescher, gesammelt und herausgegeben von seinen Freunden," Leipzig, 1897.

fat. The heads extracted with alcohol-ether contain on an average 960 p. m. protamine nucleate, which nevertheless is not uniform, but is so divided that the outer layers consist of basic protamine nucleate, while the inner layers, on the contrary, consist of acid protamine nucleate. Besides the protamine nucleate there are present in the heads, although to a very slight extent, organic substances. Of these we must mention a nitrogenous substance containing iron which gives MILLON'S reaction and which MIESCHER calls *karyogen*. The unripe salmon spermatozoa, while developing, also contain nucleic acid, but no protamine, with a protein substance, "*albuminose*," which probably is a step in the formation of protamine. According to KOSSEL and MATHEWS,[1] in the herring as in the salmon, the heads of the spermatozoa consist of protamine nucleate but no free protein.

The chemical investigations on the spermatozoa have not given us any information as to the condition for fertilization and the development of the egg.

Spermatin is a name which has been given to a constituent similar to alkali albuminate, but it has not been closely studied.

Prostatic concrements are of two kinds. One is very small, generally oval in shape, with concentric layers. In young but not in older persons they are colored blue by iodine (IVERSEN [2]). The other kind is larger, sometimes the size of the head of a pin, consisting chiefly of calcium phosphate (about 700 p.m.), with only a very small amount (about 160 p. m.) of organic substance.

(b) Female Generative Organs.

The stroma of the **ovaries** is of little interest from a physiologico-chemical standpoint, and the most important constituents of the ovaries, the *Graafian follicles* with the *ovum*, have not thus far been the subject of a careful chemical investigation. The fluid in the follicles (of the cow) does not contain, as has been stated, the peculiar bodies, paralbumin or metalbumin, which are found in certain pathological ovarial fluids, but seems to be a serous liquid. The *corpora lutea* are colored yellow. Earlier investigators (PICCOLO and LIEBEN, KÜHNE and EWALD [3]) have found a crystalline pigment in the *corpora lutea*. In recent investigations ESCHÉR [4] has shown that this substance is a crystalline hydrocarbon ($C_{40}H_{56}$) which seems to be identical with the carotin of the carrot and green leaves. The color of the crystals as well as the concentrated solution is reddish-orange. Carotin differs from the yellow pigment of the yolk of the egg, the lutein, in having another formula

[1] Zeitschr. f. physiol. Chem., **23**.
[2] Nord. med. Ark., **6**.
[3] See Chapter V, p. 301.
[4] Zeitschr. f. physiol. Chem., **83**, 198 (1912).

(page 631) and being soluble with difficulty in alcohol and readily soluble in petroleum ether.

The cysts often occurring in the ovaries are of special pathological interest, and these may have essentially different contents, depending upon their variety and origin.

The **serous cysts** (HYDROPS FOLLICULORUM GRAAFII), which are formed by a dilation of the Graafian follicles, contain a serous liquid which has a specific gravity of 1.005–1.022. A specific gravity of 1.020 is less frequent. Generally the specific gravity is lower, 1.005–1.014, with 10–40 p. m. solids. As far as is known, the contents of these cysts do not essentially differ from other serous liquids.

The **proliferous cysts** (MYXOID CYSTS, COLLOID CYSTS), which are developed from PFLÜGER'S epithelium-tubes, may have a content of a decidedly variable composition.

We sometimes find in small cysts a semi-solid, transparent, or somewhat cloudy or opalescent mass which appears like solidified glue or quivering jelly, and which has been called *colloid* because of its physical properties. In other cases the cysts contain a thick, tough mass which can be drawn out into long threads, and as this mass in the different cysts is more or less diluted with serous liquids their contents may have a variable consistency. In still other cases the small cysts may also contain a thin, watery fluid. The color of the contents is also variable. Sometimes they are bluish-white, opalescent, and again they are yellow, yellowish-brown, or yellowish with a shade of green. They are often colored more or less chocolate-brown or red-brown, due to the decomposed blood-coloring matters. The reaction is alkaline or nearly neutral. The specific gravity, which may vary considerably, is generally 1.015–1.030, but may occasionally be 1.005–1.010 or 1.050–1.055. The amount of solids is very variable. In rare cases it amounts to only 10–20 p. m.; ordinarily it varies from 50–70–100 p. m. In a few instances 150–200 p. m. solids have been found.

As form-elements one finds red and white *blood-corpuscles*, *granular cells*, partly fat-degenerated epithelium and partly large so-called GLUGE'S corpuscles, *fine granular masses*, *epithelium-cells*, *cholesterin crystals*, and *colloid corpuscles*—large, circular, highly refractive formations.

Though the contents of the proliferous cyst may have a variable composition, still it may be characterized in typical cases by its slimy or ropy consistency; by its grayish-yellow, chocolate-brown, sometimes whitish-gray color; and by its relatively high specific gravity, 1.015–1.025. Such a liquid does not ordinarily show a spontaneous fibrin coagulation.

We consider *colloid*, *metalbumin*, and *paralbumin* as characteristic constituents of these cysts.

Colloid. This name does not designate any particular chemical substance, but is given to the contents of tumors with certain physical properties similar to gelatin jelly. Colloid is found as a pathological product in several organs.

Colloid is a gelatinous mass, insoluble in water and acetic acid; it is dissolved by alkalies and gives a liquid which is not precipitated by acetic acid or by acetic acid and potassium ferrocyanide. According to PFANNENSTIEL[1] such a colloid is designated β-pseudomucin. Sometimes a colloid is found which, when treated with a very dilute alkali, gives a sclution similar to a mucin solution. Colloid is very closely related to mucin and is considered by certain investigators as a modified mucin. An ovarial colloid analyzed by PANZER contained 931 p. m. water, 57 p. m. organic substance, and 12 p. m. ash. The elementary composition was C 47.27, H 5.86, N 8.40, S 0.79, P 0.54, and ash 6.43 per cent. A colloid found by WÜRTZ[2] in the lungs contained C 48.09, H 7.47, N 7.00, and O(+S) 37.44 per cent. Colloids of different origin seem to be of varying composition.

Metalbumin. This name SCHERER[3] gave to a protein substance found by him in an ovarial fluid. The metalbumin was considered by SCHERER to be an albuminous body, but it belongs to the mucin group, and it is for this reason called *pseudomucin* by HAMMARSTEN.[4]

Pseudomucin. This body, which, like the mucins, gives a reducing substance when boiled with acids, is a mucoid of the following composition: C 49.75, H 6.98, N 10.28, S 1.25, O 31.74 per cent (HAMMARSTEN). With water pseudomucin gives a slimy, ropy solution, and it is this substance which gives the fluid contents of the ovarial cysts their typical ropy property. Its solutions do not coagulate on boiling, but only become milky or opalescent. Unlike mucin, pseudomucin solutions are not precipitated by acetic acid. With alcohol they give a coarse flocculent or thready precipitate which is soluble even after having been kept under water or alcohol, for a long time.

Paralbumin is another substance discovered by SCHERER, which occurs in ovarial liquids, and also in ascitic fluids, with the simultaneous presence of ovarial cysts and rupture of the same. It is therefore only a mixture of pseudomucin with variable amounts of protein, and the reactions of paralbumin are correspondingly variable.

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[1] Arch. f. Gynäk., 38.

[2] Panzer, Zeitschr. f. physiol. Chem., 28; Würtz, see Lebert, Beitr. zur Kenntnis des Gallertkrebses, Virchow's Arch., 4.

[3] Verh. d. physik.-med. Gesellsch. in Würzburg, 2, and Sitzungsber. der physik.-med. Gesellsch. in Würzburg für 1864–1865; Würzburg med. Zeitschr., 7, No. 6.

[4] Zeitschr. f. physiol. Chem., 6.

MITJUKOFF [1] has isolated and investigated a colloid from an ovarial cyst. It had the following composition: C 51.76, H 7.76, N 10.7 S 1.09, and O 28.69 per cent, and differed from mucin and pseudomucin by reducing FEHLING'S solution before boiling with acid. It must be remarked that pseudomucin, on boiling sufficiently long with alkali, or by the use of a concentrated solution of caustic alkali, also splits and causes a reduction. This reduction is nevertheless weak as compared with that produced after boiling with an acid. The body isolated by MITJUKOFF is called *paramucin*.

The pseudomucin as well as colloid are mucoid substances, and the carbohydrate obtained from them is glucosamine (chitosamine), as especially shown by FR. MÜLLER, NEUBERG and HEYMANN.[2] From pseudomucin ZÄNGERLE [3] obtained 30 per cent glucosamine, and NEUBERG and HEYMANN have shown that the glucosamine is the only carbohydrate regularly taking part in the structure of these substances. Still there are reports as to the occurrence of chondroitin-sulphuric acid (or an allied acid) in pseudomucin or colloid (PANZER), but this is not constant according to the experience of HAMMARSTEN.

As hydrolytic cleavage products of pseudomucin OTORI obtained, besides carbohydrate derivatives such as levulinic acid and humus substances, leucine, tyrosine, glycocoll, aspartic acid, glutamic acid, valeric acid, arginine, lysine, and guanidine. The quantity of guanidine, it seems, was greater than that which could be derived from the arginine, hence this body probably originated from another complex. PREGL [4] obtained on the hydrolysis of a colloid, which behaved like paramucin, no glycocoll and only traces of diamino acids, but otherwise the same amino-acids as OTORI found, besides alanine, proline, phenylalanine and tryptophane.

The detection of metalbumin and paralbumin is naturally connected with the detection of pseudomucin. A typical ovarial fluid containing pseudomucin is, as a rule, sufficiently characterized by its physical properties, and a special chemical investigation is necessary only in cases where a serous fluid contains very small amounts of pseudomucin. The procedure is as follows: The protein is removed by heating to boiling with the addition of acetic acid; the filtrate is strongly concentrated and precipitated by alcohol. The precipitate, a transformation product of pseudomucin, is carefully washed, with alcohol and then dissolved in water. A part of this solution is digested with saliva at the temperature of the body and then tested for glucose (derived from glycogen or dextrin). If glycogen is present, it will be converted into glucose by the saliva; precipitate again with alcohol and then proceed as in the absence of

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[1] K. Mitjukoff, Arch. f. Gynäkol., **49**.

[2] Müller, Verh. d. Naturf. Gesellsch. in Basel. **12**, part 2; Neuberg and Heymann; Hofmeister's Beiträge, **2**. See also Leathes, Arch. f. exp. Path. u. Pharm., **48**.

[3] Münch. med. Wochenschr., 1900.

[4] Otori, Zeitschr. f. physiol. Chem., **42** and **43**; Pregl, *ibid.*, **58**.

glycogen. In this last-mentioned case, first add acetic acid to the solution of the alcohol precipitate in water so as to precipitate any existing mucin. The precipitate produced is filtered off, the filtrate treated with 2 per cent HCl and warmed on the water-bath until the liquid is deep brown in color. In the presence of pseudomucin this solution gives TROMMER'S test.

The other protein bodies which have been found in cystic fluids are *serglobulin* and *seralbumin*, *peptone* (?), *mucin*, and *mucin-peptone* (?). Fibrin occurs only in exceptional cases. The quantity of mineral bodies on an average amounts to about 10 p. m. The amount of extractive bodies (*cholesterin* and *urea*) and *fat* is ordinarily 2–4 p. m. The remaining solids, which constitute the chief mass, are protein bodies and pseudomucin.

The **intraligamentary, papillary cysts** contain a yellow, yellowish-green, or brownish-green, liquid which contains either no pseudomucin or very little. The specific gravity is generally rather high, 1.032–1.036, with 90–100 p. m. solids. The principal constituents are the simple proteins of blood-serum.

The rare **tubo-ovarial cysts** contain as a rule a watery, serous fluid containing no pseudomucin.

The **parovarial cysts** or the CYSTS of the LIGAMENTA LATA may attain a considerable size. In general, and when quite typical, the contents are watery, mostly very pale-yellow-colored, water-clear or only slightly opalescent liquids. The specific gravity is low, 1.002–1.009, and the solids only amount to 10–20 p. m. Pseudomucin does not occur as a typical constituent; protein is sometimes absent, and when it does occur the quantity is very small. The principal part of the solids consists of salts and extractive bodies. In exceptional cases the fluid may be rich in protein and may show a higher specific gravity.

In regard to the quantitive composition of the fluid from ovarial cysts we refer the reader to the work of OERUM.[1]

E. LUDWIG and R. v. ZEYNEK have investigated the fat from dermoid cysts. Besides a little arachidic acid, they found oleic, stearic, palmitic, and myristic acids, cetyl alcohol, and a cholesterin-like substance. In regard to the occurrence of cetyl alcohol see the work of AMESEDER,[2] page 239.

The colloid from a uterine fibroma analyzed by STOLLMANN[3] contained a pseudomucin soluble in water, and a colloid (paramucin) insoluble in water, both of which behaved differently with alcohol as compared with the corresponding substances from ovarial cysts.

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[1] Kemiske Studier over Ovariecystevædsker, etc., Koebenhavn, 1884. See also Maly's Jahresber., **14**, 459.

[2] Ludwig and v. Zeynek, Zeitschr. f. physiol. Chem., **23**; Ameseder, *ibid.*, **52**; Salkowski, Bioch. Zeitschr., **32**.

[3] Amer. Gynecology, 1903.

The Ovum.

The small ova of man and mammals cannot, for evident reasons, be the subject of a searching chemical investigation. Up to the present time the eggs of birds, amphibians, and fishes have been investigated, but above all the hen's egg. We will here occupy ourselves with the constituents of this last.

The Yolk of the Hen's Egg. In the so-called white yolk, which forms the *germ* with a process reaching to the center of the yolk (*latebra*), and forming a layer between the yolk and yolk-membrane, there occurs *protein*, *nuclein*, *lecithin*, and *potassium* (LIEBERMANN [1]). The occurrence of glycogen is doubtful. The yolk-membrane consists of an albuminoid similar in certain respects to keratin (LIEBERMANN).

The principal part of the yolk—the nutritive yolk or yellow—is a viscous, non-transparent, pale-yellow or orange-yellow alkaline emulsion of a mild taste. The yolk contains *vitellin*, *lecithin*, *cholesterin*, *fat*, *coloring-matters*, traces of *neuridine* (BRIEGER [2]), *purine bases* (MESERNITZKI [3]), *glucose* in very small quantities, and *mineral bodies*. The occurrence of cerebrin and of granules similar to starch (DARESTE [4]) has not been positively proved.

Several enzymes have been found in the yolk, especially a diastatic enzyme (MÜLLER and MASUYAMA), a glycolytic enzyme (STEPANEK) which in the absence of air brings about an alcoholic fermentation of sugar and in the presence of air forms carbon dioxide and lactic acid, and finally a proteolytic, a lipolytic, and a chromolytic (?) enzyme (WOHLGEMUTH [5]).

Ovovitellin. This body, which is often considered as a globulin is in reality a nucleoalbumin. The question as to what relation other protein substances, which are related to ovovitellin, like the *aleuron grains* of certain seeds, and the *yolk spherules* of the eggs of certain fishes and amphibians, bear to this substance is one which requires further investigation.

The ovovitellin which has been prepared from the yolk of eggs is not a pure protein body, but always contains lecithin. HOPPE-SEYLER found 25 per cent lecithin in vitellin. The lecithin may be removed by boiling alcohol, but the vitellin is changed thereby, and it is therefore probable

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[1] Pflüger's Arch., **43**.

[2] Ueber Ptomaine, Berlin, 1885.

[3] Mesernitzki, Biochem. Centralbl., **1**, 739.

[4] Compt. Rend., **72**.

[5] Müller and Masuyama, Zeitschr. f. Biologie, **39**; Stepanek, Centralbl. f. Physiol., 18, 188; Wohlgemuth in Salkowski's Festschrift and Zeitschr. f. physiol. Chem., **44**.

that the lecithin is chemically united with the vitellin (HOPPE-SEYLER [1]). According to OSBORNE and CAMPBELL, the so-called ovovitellin is a mixture of various vitellin-lecithin combinations, with 15 to 30 per cent of lecithin. The protein substance freed from lecithin is the same in all these compounds and has the following composition: C 51.24, H 7.16, N 16.38, S 1.04, P 0.94, O 23.24 per cent. These figures differ somewhat from those obtained by GROSS for vitellin prepared by another method (precipitation with $[NH_4]_2SO_4$), namely, C 48.01, H 6.35, N 14.91–16.97, P 0.32–0.35, S 0.88, and the composition of ovovitellin is therefore not positively known. Besides the vitellin GROSS found a globulin coagulating at 76–77° C. in a solution containing salt, and PLIMMER [2] found a protein which he calls *livetin* which only contained 0.1 per cent phosphorus and which gave more monamino acids but less amide and diamino nitrogen than vitellin.

On the pepsin digestion of ovovitellin, OSBORNE and CAMPBELL obtained a pseudonuclein with varying amounts of phosphorus, 2.52–4.19 per cent. BUNGE [3] prepared a pseudonuclein by digesting the yolk with gastric juice, and his pseudonuclein, he claims, is of great importance in the formation of the blood, and on these grounds he called it *hæmatogen*. This hæmatogen has the following composition: C 42.11, H 6.08, N 14.73, S 0.55, P 5.19, Fe 0.29, and O 31.05 per cent. The composition of this substance may vary considerably even on using the same method of preparation.

Vitellin is similar to the globulins in that it is insoluble in water, but on the contrary soluble in dilute neutral-salt solutions (although the solution is not quite transparent). It is also soluble in hydrochloric acid of 1 p. m. and in very dilute solutions of alkalies or alkali carbonates. It is precipitated from its salt solution by diluting with water, and when allowed to stand some time in contact with water the vitellin is gradually changed, forming a substance more like the albuminates. The coagulation temperature for the solution containing salt (NaCl) lies between 70 and 75° C., or, when heated very rapidly, at about 80° C. Vitellin differs from the globulins in yielding pseudonuclein by peptic digestion. It is not always completely precipitated by NaCl in substance. The ovovitellin isolated by GROSS gave MOLISCH'S reaction. NEUBERG [4] has also split off glucosamine from the yolk and has identified it as nori-

[1] Med. chem. Untersuch., 216.

[2] Osborne and Campbell, Connecticut Agric. Exp. Station, 23d Ann. Report, New Haven, 1900; Gross, Zur Kenntn. d. Ovovitellins, Inaug.-Diss. Strassburg, 1899; Plimmer, Journ. Chem. Soc., London, **93**.

[3] Zeitschr. f. physiol. Chem., **9**, 49. See also Hugounenq and Morel, Compt. Rend., **140** and **141**.

[4] Ber. d. d. chem. Gesellsch., **34**.

sosaccharic acid. It is difficult to state whether this glucosamine was derived from the vitellin or from some other constituent of the yolk.

The principal points in the preparation of ovovitellin are as follows: The yolk is thoroughly agitated with ether; the residue is dissolved in a 10-per cent common-salt solution, filtered, and the vitellin precipitated by adding an abundance of water. The vitellin is now purified by repeatedly redissolving in dilute common-salt solutions and precipitating with water.

Ichthulin, which occurs in the eggs of the carp and other fishes is, according to KOSSEL and WALTER, an amorphous modification of the crystalline body *ichthidin*, which occurs in the eggs of the carp. Ichthulin is precipitated on diluting with water. It was formerly considered as a vitellin. According to WALTER it yields a pseudonuclein on peptic digestion; and this pseudonuclein gives a reducing carbohydate on boiling with sulphuric acid. Ichthulin has the following composition; C 53.42, H 7.63, N 15.63, O 22.19, S 0.41, P 0.43 per cent. It also contains iron. The ichthulin investigated from codfish eggs by LEVENE had the composition C 52.44, H 7.45, N 15.96, S 0.92, P 0.65, Fe+O 22.58 per cent, and yielded no reducing substances on boiling with acids. The pure vitellin isolated by HAMMARSTEN from perch eggs had a similar behavior and was very readily changed by a little hydrochloric acid so that it was converted into a typical pseudonuclein. The codfish ichthulin yielded a pseudonucleic acid with 10.34 per cent phosphorus, but this acid still gave the protein reactions. MCCLENDEN [1] has prepared a vitellin from frogs' eggs which he calls *batrachiolin*.

The yolk also contains *albumin*, besides vitellin and the above-mentioned proteins.

The *fat* of the yolk of the egg, LIEBERMANN [2] claims, is a mixture of a solid and a liquid fat. The solid fat consists principally of tripalmitin with some tristearin. On the saponification of the egg-oil LIEBERMANN obtained 40 per cent oleic acid, 38.04 per cent palmitic acid, and 15.21 per cent stearic acid. The fat of the yolk of the egg contains less carbon than other fats, which may depend upon the presence of monoglycerides and diglycerides, or upon a quantity of fatty acid deficient in carbon (LIEBERMANN). The composition of yolk fat is dependent upon the food, as HENRIQUES and HANSEN [3] have shown that the fat of the food passes into the egg.

The phosphatides of the yolk seem to be of various kinds. THIERFELDER and STERN have found three different phosphatides. One of these, which was soluble in alcohol-ether, behaved like lecithin. The second was soluble with difficulty in alcohol, but readily soluble in ether, contained 1.37 per cent N and 3.96 per cent P. The third was a diamino

[1] Walter, Zeitschr. f. physiol. Chem., **15**; Levene, *ibid.*, **32**; Hammarsten, Skand. Arch. f. Physiol., **17**; McClenden, Amer. Journ. of Physiol. **25**; see also Plimmer and Scott, Journ. Chem. Soc., **93**.

[2] Pflüger's Arch., **43**.

[3] Skand. Arch. f. Physiol., **14**.

phosphatide, soluble with difficulty in ether, but obtained in crystalline needles from hot alcohol, and contained 2.77 per cent N and 3.22 per cent P, and had a melting-point of 160–170° C. FRÄNKEL and BOLAFFIO [1] also found a substance crystallizing from hot alcohol and insoluble in ether with 2.78 per cent N and 2.18 per cent P. They call this body *neottin* and claim that it is a triamino-monophosphatide having the formula $C_{84}H_{172}N_3PO_{15}$. BARBIERI has obtained a sulphurized phosphatide called *ovin*, containing 1.35 per cent P, 3.66 per cent N and 0.4 per cent S. The relation of all these bodies to each other must be further studied.

Lutein. With the name lutein we in the past have included several yellow or orange-red amorphous coloring-matters which occur in the yellow of the egg, and in several other places in the animal organism; for instance, in the blood-serum and serous fluids, fatty tissues, milk-fat, *corpora lutea*, and in the fat-globules of the retina as well as in different plants (THUDICHUM). Among these bodies belong the crystalline substance obtained by ESCHER from the *corpora lutea* (page 623). It was difficultly soluble in alcohol but readily soluble in petroleum ether and showed itself isomeric or perhaps identical with the plant pigment carotin ($C_{40}H_{56}$) analyzed by WILLSTÄTTER and MIEG. The lutein of the egg yolk, which is more readily soluble in alcohol and less soluble in petroleum ether than carotin has also been obtained by WILLSTÄTTER and ESCHER in a pure, crystalline form. On analysis it gave the formula $C_{40}H_{56}O_2$. As shown by C. A. SCHUNCK the yolk lutein stands in close relation to the yellow plant pigment, *xanthophyll*. The formula given by WILLSTÄTTER and ESCHER for lutein was in fact the same as for the xanthophyll, as previously found by WILLSTÄTTER and MIEG. These two substances are also similar in other respects; still the melting-points of the two are different. The carotin and the yolk lutein differ also by the absorption spectra, which is different in different solvents as well as by their formulæ and different solubilities.[2]

The relation of the other substances called luteins to each other and to the yolk lutein is unknown. All are soluble in alcohol, ether, and chloroform. They differ from the bile-pigment, bilirubin, in that they are not separated from their solution in chloroform by water containing alkali, and also in that they do not give the characteristic play of colors with nitric acid containing a little nitrous acid, but give a transient blue color. The luteins withstand the action of alkalies so that they are not changed when we remove the fats present by means of saponification.

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[1] Thierfelder and Stern, Zeitschr. f. physiol. Chem., **53**; Fränkel and Bolaffio, Bioch. Zeitschr., **9**; Barbieri, Compt. Rend., **145**.

[2] Thudichum, Centralbl. f. d. med. Wiss. 1869; Willstätter and Mieg. Ann. d. Chem., **355** (1907); Willstätter and Escher, Zeitschr. f. physiol. Chem., **64** (1909); **76** (1911); Schunck, see Chem. Centralbl., 1903.

MALY [1] found two pigments free from iron in the eggs of a water-spider (Maja squinado)—one a red (*vitellorubin*) and the other a yellow pigment (*vitellolutein*). Both of these pigments are colored blue by nitric acid containing nitrous acid and a beautiful green by concentrated sulphuric acid.

The *mineral bodies* of the yolk of the egg consist, according to POLECK,[2] of 51.2–65.7 parts soda, 80.5–89.3 potash, 122.1–132.8 lime, 20.7–21.1 magnesia, 11.90–14.5 iron oxide, 638.1–667.0 phosphoric acid, and 5.5–14.0 parts silicic acid in 1000 parts of the ash. We find phosphoric acid and lime the most abundant, and then potash, which is somewhat greater in quantity than the soda. These results are not, however, quite correct: first, because no dissolved phosphate occurs in the yolk (LIEBERMANN), and secondly, in burning, phosphoric and sulphuric acids are produced, and these drive away the chlorine, which is not accounted for in the above analyses.

The yolk of the hen's egg weighs about 12–18 grams. The quantity of water and solids amounts, according to PARKE,[3] to 471.9 p. m. and 528.1 p. m. respectively. Among the solids he found 156.3 p. m. protein, 3.53 p. m. soluble and 6.12 p. m. insoluble salts. The quantity of fat, according to PARKE, is 228.4 p. m.; the lecithin, calculated from the amount of phosphorus in the organic substance of the alcohol-ether extract, was 107.2 p. m. and the cholesterin 17.5 p. m.

The **white of the egg** is a faintly yellow albuminous fluid inclosed in a framework of thin membranes; and this fluid is in itself very liquid, but seems viscous because of the presence of these fine membranes. That substance which forms the membranes, and of which the *chalaza* consists, seems to be a body closely related to horn substances (LIEBERMANN).

The white of egg has a specific gravity of 1.038–1.045, and always has an alkaline reaction toward litmus. It contains 850–880 p. m. water, 100–130 p. m. protein bodies, and 7 p. m. salts. LEHMANN found a fermentable *variety of sugar* which SALKOWSKI showed was glucose. C. TH. MÖRNER could not find any other sugar in egg-white; the quantity of glucose as found by MÖRNER[4] was 3–5 p. m. Besides these one finds in the white of egg traces of fats, soaps, lecithin and cholesterin.

The white of egg of the Insessores becomes transparent on boiling and acts in many respects like alkali albuminate. This albumin TARCHANOFF [5] called "*tatalbumin.*"

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[1] Monatshefte f. Chem., **2**.

[2] Cited from v. Gorup-Besanez, Lehrbuch d. physiol. Chem., **4**, Aufl., **740**.

[3] Hoppe-Seyler, Med. chem. Untersuch., Heft 2, 209.

[4] Lehmann, Lehrb. d. physiol. Chem. 2 Aufl. 1855, Bd. 1, s. 271; Bd. 2, s. 312. Salkowski, Centralbl. f. d. med. Wiss., **31** (1893); Mörner, Zeitschr. f. physiol. Chem; **80** (1912).

[5] Pflüger's Arch., **31**, **33**, and **39**.

The protein substances of the white of egg behave like glycoproteins, as they all yield glucosamine. For the globulin and albumin it has not been proved, nor is it probable, that the glucosamine belongs to the protein molecule (see page 84). According to the solution and precipitation properties they are similar to the globulins, albumins or proteoses. The representatives of the first two groups, are *ovoglobulin* and *ovalbumin*. The proteose-like body is *ovomucoid*.

Ovoglobulin separates in part on diluting the egg-white with water. It is precipitated upon saturation with magnesium sulphate, or upon one-half saturation with ammonium sulphate, and coagulates at about 75° C. By repeated solution in water and precipitation with ammonium sulphate a part of the globulin becomes insoluble (LANGSTEIN). This also occurs on precipitation by diluting with water or by dialysis, and it is quite possible that the globulin is a mixture. That portion which readily becomes insoluble seems to be identical with EICHHOLZ's glycoprotein or OSBORNE and CAMPBELL's ovomucin. LANGSTEIN obtained 11 per cent of glucosamine from the soluble ovoglobulin. The total quantity of globulins, according to DILLNER, is about 6.7 per cent of the total protein substances, and this corresponds with the recent determinations of OSBORNE and CAMPBELL. In regard to the probable occurrence of several globulins in the white of the egg there are the determinations of CORIN and BERARD as well as of LANGSTEIN,[1] but they have not led to any positive conclusions.

Ovalbumin. The so-called albumin of the egg-white is undoubtedly a mixture of at least two albumin-like proteins. Opinions differ considerably in regard to the number of these proteins (BONDZYNSKI and ZOJA, GAUTIER, BÉCHAMP, CORIN and BERARD, PANORMOFF, and others). Since HOFMEISTER has been able to prepare ovalbumin in a crystalline form, and since HOPKINS and PINKUS [2] have shown that not more than one-half of the ovalbumin can be obtained in such a form, OSBORNE and CAMPBELL have isolated two different ovalbumins or principal fractions; the crystallizable they call *ovalbumin* and the non-crystallizable *conalbumin*. The two fractions have only a slight variation in elementary composition; the conalbumin coagulates between 50–60° C., nearer to 60° C., and the ovalbumin at 64° C. or at a higher temperature. There are no conclusive investigations as to whether the non-crystallizable

[1] Langstein, Hofmeister's Beiträge, **1**; Eichholz, Journ. of Physiol., **23**; Osborne and Campbell, Connecticut Agric. Exp. Station., 23d Ann. Report, New Haven, 1900; Dillner, Maly's Jahresber., **15**; Corin and Berard, *ibid.*, **18**.

[2] Hofmeister, Zeitschr. f. physiol. Chem., **14**, **16**, and **24**, Gabriel, *ibid.*, **15**; Bondzynski and Zoja, *ibid.*, **19**; Gautier, Bull. Soc. chim., **14**; Béchamp, *ibid.*, **21**; Corin and Berard, l. c.; Hopkins and Pinkus, Ber. d. d. chem. Gesellsch., **31**, and Journ. of Physiol., **23**; Osborne and Campbell, l. c.; Panormoff, Maly's Jahresber., **27** and **28**.

conalbumin is a mixture or not, and the question concerning the unity of the crystallizable ovalbumin is also disputed. According to BONDZYNSKI and ZOJA, crystallizable ovalbumin is a mixture of several albumins having somewhat different coagulation temperatures, solubilities, and specific rotations, while HOFMEISTER and LANGSTEIN on the contrary believe that crystallizable ovalbumin is a unit. The reports as to the specific rotation of the different fractions unfortunately differ, and the elementary analyses have also given no positive results, as a variation of 1.2–1.7 per cent has been observed in the quantity of sulphur. According to the consistent analyses of OSBORNE and CAMPBELL and of LANGSTEIN, the conalbumin contains about 1.7 per cent sulphur and about 16 per cent nitrogen, while the ovalbumin contains on an average about 15.3 per cent nitrogen. LANGSTEIN [1] obtained 10–11 per cent glucosamine from ovalbumin and about 9 per cent from conalbumin. The ovalbumin, like the conalbumin, has the properties of the albumins in general, but differs from seralbumin in that the specific rotation is lower. It is quickly made insoluble by alcohol and is precipitated by a sufficient quantity of HCl, but dissolves in an excess of acid with greater difficulty than the seralbumin. The products isolated by ABDERHALDEN and PREGL [2] on the hydrolysis of ovalbumin do not show anything of special interest.

As in the past certain doubts have existed as to the purity and chemical unity of the ovalbumins, or also of the crystalline ovalbumin, so now this doubt has become still stronger since ovalbumin has been prepared partly free from phosphorus and partly with a variable phosphorus content of 0.1–3.06 per cent (KAAS, WILLCOCK and HARDY [3]).

In preparing crystalline ovalbumin, mix, according to HOFMEISTER, the beaten white of egg free from foam with an equal volume of a saturated ammonium-sulphate solution, filter off the globulin, and allow the filtrate to evaporate slowly in thin layers at the temperature of the room. After a time the masses which separate out are dissolved in water, treated with ammonium sulphate-solution until they begin to get cloudy, and are allowed to stand. After repeated recrystallization the mass is either treated with alcohol, which makes the crystals insoluble, or they are dissolved in water and purified by dialysis. From these solutions the proteid does not crystallize again on spontaneous evaporation. (See also page 633, footnote 2, for the HOPKINS and PINKUS method.) WILLCOCK [4] has recently found that magnesium sulphate can also be used in the crystallization of ovalbumin.

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[1] Zeitschr. f. physiol. Chem., **31**.

[2] *Ibid.*, **46**.

[3] Kaas, Monatsh. f. Chem., **27**; Willcock and Hardy, cited from Chem. Centralbl. 1907, **2**, 821.

[4] Journ. of Physiol., **37**.

Conalbumin can be removed from the filtrate, after the complete crystallization of the ovalbumin, by removing the sulphate by means of dialysis and coagulating by heat.

GAUTIER [1] found a fibrinogen-like substance in the white of egg, which was changed into a fibrin-like body by the action of a ferment.

Ovomucoid. This substance, first observed by NEUMEISTER and considered by him as a pseudopeptone, and then later studied by SALKOWSKI, is, according to C. TH. MÖRNER,[2] a mucoid with 12.65 per cent nitrogen and 2.20 per cent sulphur. Ovomucoid exists in hens' eggs to the extent of about 12 per cent of the total solids.

A solution of ovomucoid is not precipitated by mineral acids nor by organic acids, with the exception of phosphotungstic acid and tannic acid. It is not precipitated by metallic salts, but basic lead acetate and ammonia render it insoluble. Ovomucoid is thrown down by alcohol, but sodium chloride, sodium sulphate, and magnesium sulphate give no precipitates either at the ordinary temperature or when the salts are added to saturation at 30° C. Its solutions are not precipitated by an equal volume of a saturated solution of ammonium sulphate, but are precipitated on adding more salt thereto. The substance is not precipitated on boiling, but the part which has become insoluble in cold water and which has been dried, is dissolved by boiling water. ZANETTI has prepared glucosamine on splitting ovomucoid with concentrated hydrochloric acid, and SEEMANN found that the quantity of glucosamine in ovomucoid was 34.9 per cent.[3]

Ovomucoid may be prepared by removing all the proteins by boiling with the addition of acetic acid, and then concentrating the filtrate and precipitating with alcohol. The substance is purified by repeated solution in water and precipitation with alcohol.

PANORMOW believes that the eggs of other birds, such as the pigeon and duck, contain a special protein in the egg-white, which is not identical with that of the hen's egg. WORMS [4] has prepared a crystalline albumin from the white of the turkey eggs which contained 15.37 per cent N, 1.6 per cent S and had a specific rotation of $(\alpha)_D = -34.9°$.

The *mineral bodies* of the white of egg have been analyzed by POLECK and WEBER.[5] They found in 1000 parts of the ash: 276.6–

[1] Compt. Rend., 185.

[2] R. Neumeister, Zeitschr. f. Biologie, 27; Salkowski. Centralbl. f. d. med. Wissensch., 1893, 513 and 706; C. Mörner, Zeitsch. f. physiol. Chem., 18 and 80. See also Langstein, Hofmeister's Beiträge, 3 (literature).

[3] Zanetti, Chem. Centralbl., 1898, 1; Seemann, cited from Langstein, Ergebnisse der Physiol., 1, Abt. 1, 86.

[4] Panormow, see Bioch. Centralbl., 5; Worms, cited from Chem. Centralbl., 1906, 2, 1508.

[5] Cited from Hoppe-Seyler, Physiol. Chem., 778.

284.5 grams potash, 235.6–329.3 soda, 17.4–29.0 lime, 17–31.7 magnesia, 4.4–5.5 iron oxide, 238.4–285.6 chlorine, 31.6–48.3 phosphoric acid (P_2O_5), 13.2–26.3 sulphuric acid, 2.8–20.4 silicic acid, and 96.7–116.0 grams carbon dioxide. Traces of fluorine have also been found (NICKLÉS [1]). The white of egg contains, as compared with the yolk, a greater amount of chlorine and alkalies and a smaller amount of lime, phosphoric acid, and iron.

The Shell-membrane and the Egg-shell. The shell-membrane consists, as above stated (page 112), of a keratin substance. The shell contains very little organic substance, 36–65 p. m. The principal mass, more than 900 p. m., consists of calcium carbonate; besides this there are very small amounts of magnesium carbonate and earthy phosphates.

The diverse *coloring* of birds' eggs is due to several different coloring-matters. Among these we find a red or reddish-brown pigment called "*oorodein*" by SORBY,[2] which is perhaps identical with hæmatoporphyrin. The green or blue coloring-matter, SORBY'S *oocyan*, seems, according to LIEBERMANN [3] and KRUKENBERG,[4] to be partly *biliverdin* and partly a blue *derivative of the bile-pigments*.

The eggs of birds have a space at their blunt end filled with gas; this gas contains on an average 18.0–19.9 per cent oxygen (HÜFNER [5]).

The weight of a hen's egg varies between 40–60 grams and may sometimes reach 70 grams. The shell and shell-membrane together, when carefully cleaned, but still in the moist state, weigh 5–8 grams. The yolk weighs 12–18 and the white 23–34 grams, or about double. The entire egg contains 2.8–7.5, or average 4.6, milligrams of iron oxide, and the quantity of iron can be increased by food rich in iron (HARTUNG [6]).

The white of the egg of cartilaginous and bony fishes contains only traces of true albumin, but consists, at least in many fishes, of mucin substance; and the cover of the frog's egg also consists, according to GIACOSA, of mucin. The eggs of the river-perch contain, HAMMARSTEN [7] claims, mucin in the envelope in the unripe state and only mucinogen in the ripe state. The crystalline formations (*yolk-spherules*, or *dotterplättchen*) which have been observed in the egg of the tortoise, frog, ray, shark, and other fishes, and which are described by VALENCIENNES and FRÉMY under the names *emydin*, *ichthin*, *ichthidin*, and *ichthulin*, seem, as above stated in connection with ichthulin, to consist mainly of phosphoglycoproteins. The *klupeovin* obtained by HUGOUNENQ [8] from the herrings' eggs and from which he obtained the three so-called hexone bases and abundant

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[1] Compt. Rend., **43**.

[2] Cited from Krukenberg, Verh. d. phys.-chem. Gesellsch. in Würzburg, **17**.

[3] Ber. d. deutsch. chem. Gesellsch., **11**.

[4] l. c.

[5] Arch. f. (Anat. u). Physiol., 1892.

[6] Zeitschr. f. Biol., **43**.

[7] Giacosa, Zeitschr. f. physiol. Chem., **7**; Hammarsten, Skand. Arch. f. Physiol., **17**.

[8] Valenciennes and Frémy. cited from Hoppe-Seyler, Physiol. Chem., p. **77**; Hugounenq, Bull. soc. chim. (3), **33**, and Compt. Rend., **143**.

monamino-acids, especially leucine, but not glycocoll or glutamic acid, is to all appearances not a unit body. The eggs of the river-crab and the lobster contain the same pigment as the shell of the animal. This pigment, called *cyanocrystallin*, becomes red on boiling in water.

C. MÖRNER [1] has isolated a substance which he calls *percaglobulin*, from the unripe eggs of the river-perch. It is a globulin and has a strong astringent taste. Especially striking is its property of precipitating certain glycoproteins, such as ovomucoid and ovarial mucoids, and polysaccharides, such as glycogen, gum, tragacanth and starch-paste, and of being precipitated by them. Percaglobulin could not be obtained by MÖRNER from the eggs of the sea-bass.

In fossil eggs (of APETNODYTES, PELECANUS, and HALLÆUS) in old guano deposits, a yellowish-white, silky, laminated compound has been found which is called *guanovulit*, $(NH_4)_2SO_4+2K_2SO_4+3KHSO_4+4H_2O$, and which is easily soluble in water, but is insoluble in alcohol and ether.

Those eggs which develop outside of the mother-organism must contain all the elements necessary for the young animals. One finds, therefore, in the yolk and white of the egg an abundant quantity of protein bodies of different kinds, and especially phosphorized proteins in the yolk. Further, we also find abundance of phosphatides in the yolk, which seem to occur habitually in all developing cells. KATO and BLEIBTREU [2] found glycogen in the eggs of the frog which during the spawning season increased at the cost of the liver glycogen. Besides this the egg is very rich in fat, which doubtless is important as a source of supply for nourishment and in maintaining respiration for the embryo. The cholesterin or at least the lutein can hardly have a direct influence on the development of the embryo. The egg also seems to contain the mineral bodies necessary for the development of the young animal. The lack of phosphoric acid is compensated by an abundant amount of phosphorized organic substance, and the nucleoalbumin containing iron, from which the hæmatogen (see page 629) is formed, is doubtless, as BUNGE claims, of great importance in the formation of the hæmoglobin containing iron. The silicic acid, necessary for the development of the feathers, is also found in the egg.

During the period of incubation the egg loses weight, due chiefly to loss of water. The quantity of solids, especially the fat and the proteins, diminishes, and the egg gives off carbon dioxide, but TANGL disproves the older claim of LIEBERMANN [3] that nitrogen or a nitrogenous substance is given off. On the contrary a corresponding absorption of oxygen takes place, and it is found that during incubation a respiratory exchange of gases occurs.

As BOHR and HASSELBALCH have shown by exact investigations, the elimination of carbon dioxide is very small in the first days of incuba-

[1] Zeitschr. f. physiol. Chem., **40** and **58**.

[2] Kato, Pflüger's Arch. **132**; Bleibtreu, *ibid.*, **132** (1910).

[3] Tangl and v. Mituch, Pflüger's Arch., **121**; Liebermann, *ibid.*, **43**.

tion; on the fourth day the carbon-dioxide production gradually increases, and after the ninth day it augments in the same proportion as the weight of the fœtus. Calculated upon 1 kilogram weight for one hour it is, from the ninth day on, about the same as in the full-grown hen. HASSELBALCH [1] has also shown that the fertilized hen's egg not only gives off nitrogen the first five or six hours of incubation, but also some oxygen, and that we are here dealing with an oxygen production which runs parallel with the cell-division. It is not known whether this oxygen formation connected with the life of the cell is a fermentative or a so-called vital process.

While the quantity of dry substance in the egg during this period always decreases, the quantity of mineral bodies, protein, and fat always increases in the embryo. The increase in the amount of fat in the embryo depends, in great part upon a taking up of the nutritive yolk in the abdominal cavity. PLIMMER and SCOTT [2] have observed in the incubation of the hen's egg, that a rapid diminution of phosphorized substances soluble in ether takes place, while at the same time an increase in the inorganic phosphorus is found in the chick.

The weight of the shell and the quantity of lime-salts contained therein do not remain unchanged, according, to the recent investigations of TANGL.[3] The egg-shell (lime shell, and shell-membrane) of a hen's egg weighing 60 grams loses (calculated on the dry) during incubation about 0.4 gram, of which 0.15 gram is calcium and 0.2 gram is organic substance.

A very complete and careful chemical investigation on the development of the embryo of the hen has been made by LIEBERMANN.[4] From his researches we may quote the following: In the earlier stages of the development, tissues very rich in water are formed, but upon the continuation of the development the quantity of water decreases. The absolute quantity of the bodies soluble in water increases with the development, while their relative quantity, as compared with the other solids, continually decreases. The quantity of the bodies soluble in alcohol quickly increases. A specially important increase is noticed in the fat, whose quantity is not very great even on the fourteenth day, but after that it becomes considerable. The quantity of protein bodies and albuminoids soluble in water grows continually and regularly in such a way that their absolute quantity increases, while their relative quantity remains nearly unchanged. LIEBERMANN found no gelatin in the

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[1] Bohr and Hasselbalch, Maly's Jahresber., 29; Hasselbalch, Skand. Arch. f. Physiol., 13.

[2] Journ. of Physiol., 38.

[3] Tangl with Hammerschlag, Pflüger's Arch., 121.

[4] l. c.

embryo of the hen. The embryo does not contain any gelatin-forming substance until the tenth day, and from the fourteenth day on it contains a body which, when boiled with water, gives a substance similar to chondrin. A body similar to mucin occurs in the embryo when about six days old, but then disappears. The quantity of hæmoglobin shows a continual increase compared with the weight of the body. LIEBERMANN found that the relation of the hæmoglobin to the body weight was 1:728 on the eleventh day and 1:421 on the twenty-first day.

By means of BERTHELOT'S thermometric methods TANGL[1] has determined the chemical energy present at the beginning and end of the development of the embryo of the sparrow's and hen's eggs. The difference was considered as work of development. He found that the chemical energy necessary for the development of each gram of ripe hen's embryo (Plymouth) was equal to 0.805 Cal. This energy originated chiefly from the fat. Of the total chemical energy utilized, about 70 per cent was used for the embryo and about 30 per cent remained in the yolk. Of the utilized energy about two-thirds was used in the construction of the embryo and about one-third transformed into other forms of energy as work of development.

By their investigations on the development of the trout egg, TANGL and FARKAS[2] have found that the loss in weight of each egg which had an average weight of 88 milligrams was 4.9 milligrams during the 42 days of incubation, of which 4.11 milligrams was water and 0.722 milligram dry substance with 0.367 milligram C. The eggs lose no nitrogen and no fat. The fat content increases a little, and indeed, as these authors believe, at the expense of the proteins. The chemical energy used during development was 6.68 gram-calories.

The highly interesting investigations made by LOEB upon the fertilization of the eggs of lower sea-animals will be discussed in this connection. According to these experiments after the fertilization of the egg by means of a sort of cytolysis small drops of a colloid substance form on the surface of the egg. These drops enlarge in volume and conglomerate to a continuous mass, while its surface hardens to a tight, continuous membrane—the fertilization membrane. The process of membrane formation is in fact the essential step in the fertilization. Besides, by spermatozoa, the membrane formation is caused by different actions. For many eggs all that is necessary is the artificial calling forth of the processes for the membrane formation in order that the egg shall develop to normal larvæ (for example the eggs of the star fish and of certain worms). In other cases, for example the sea-urchin, Strongylocentrotus, a second action is necessary for the production of

[1] Pflüger's Arch., **98** and **121**.

[2] *Ibid.*, **104**.

normal larvæ. The principal points in the treatment of such eggs are the following.

The formation of the fertilization membrane can be brought about by placing the eggs in sea water which has been faintly acidified with a fatty acid, for example with butyric acid, and after 1½ to 2 minutes placed again in sea-water. The formation of the membrane now takes place. The oxyacids and especially the inorganic acids are less active. than the fatty acids. The H-ions are without effect in this acid action and LOEB explains the action by the introduction of the undissociated molecules into the egg. Parallel with the membrane formation chemical processes begin, among which we must especially mention oxidations. These processes, if they proceed undisturbed, especially at 15° or above, lead quickly to the death of the egg. This can, nevertheless, be prevented if the oxidation processes are inhibited 40–60 minutes after the membrane formation by removing the oxygen or by the addition of some potassium cyanide. In this process probably certain injurious substances for the egg are destroyed. If eggs treated in this way are placed in sea-water after 2–3 hours they develop in a normal manner.

The membrane formation can also be brought about in other ways besides by the action of acids, for example by treating the egg with saponin, solanin, digitalin, soaps and fat dissolving substances such as amylene, benzene, toluene, chloroform, ether and alcohol. The sea-urchin egg is also excited to membrane formation by the serum of certain animals. Alkalies and elevation of temperature can also cause the formation of membrane.

On the other hand the chemical processes, which, when not prevented, lead to the death of the egg, can also be inhibited by placing the eggs in a hypertonic solution (50 cc. sea-water and 8 cc. 2.5 normal NaCl) about one hour after the artificial membrane has been formed and then after 20–50 minutes placing them in sea-water again.

According to LOEB the artificial fertilization of the sea-urchin's egg depends upon two special actions, of which the first brings about the formation of membrane with oxidation processes by means of cytolysis while the second gives the direction of these oxidation processes necessary for the maintenance of life.

The non-fertilized, ripe egg, as the investigations of LOEB on starfish have shown, dies in 4–6 hours at sufficiently high temperatures. The death of the egg can, nevertheless, be prevented if oxygen is removed from the egg or the oxidation inhibited by the addition of traces of potassium cyanide. If the ripe egg is fertilized by spermatozoa then it remains alive although the process of fertilization, as WARBURG[1] found, causes

[1] Zeitschr. f. physiol. Chem., **57**, **60**, **66**.

a considerable rise in the oxidation. For this reason LOEB believes that the spermatozoa save the life of the egg by bringing membrane forming substances to the egg, but also other substances, which remove or make inert a harmful substance or condition complex of the unfertilized egg, so that even now the increased oxidation cannot have any harmful effect.[1]

The enzymes of the sea-urchin suffer an increase in natural as well as in artificial fertilization as JACOBY[2] has shown that glycyltryptophane is split after fertilization but not before.

The **placenta** has recently been the subject of several investigations. This tissue contains a protein which coagulates at 60–65° C. (BOTTAZZI and DELFINO) whose relation to the *nucleoprotein*, found by others, is not clear. The protein found by SAVARÉ contained 0.45 per cent phosphorus. The nucleic acid studied by KIKKOJI,[3] which is very similar to the thymus nucleic acid, originates from this nucleoprotein. *Glycogen* occurs regularly in the placenta, and MOSCATI believes the human placenta contains 5 p. m. glycogen. After removal the glycogen diminishes, and after 24 hours it has disappeared. According to LOCHHEAD and CRAMER[4] the quantity of glycogen in the placenta is not increased by food rich in carbohydrate. In the fœtus (rabbits) the above authors found that the placenta is a storage organ for glycogen until the second half of the gestation period, when the liver begins to functionate in this direction. From this time on the quantity of glycogen in the placenta diminishes.

Enzymes of various kinds, proteolytic as well as lipolytic (monobutyrase), amylases and oxidases have been found in the placenta.[5] In the edges of the placenta of the bitch and of cats, an orange-colored, crystalline pigment (bilirubin) and a green, amorphous pigment, whose relation to biliverdin is not clear, have been found.[6]

From the cotyledons of the placenta in ruminants a white or faintly rose-colored creamy fluid, the *uterine milk*, can be obtained by pressure. It is alkaline in

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[1] A complete review of the investigations of Loeb and his collaborators, with the literature can be found in Vorlesungen über die Dynamik der Lebenserscheinungen, Leipzig, 1906, s. 239. See also Über den chemischen Charakter des Befruchtungsvorganges, Leipzig, 1908; Zeitschr. f. physik. Chem. **70**, 220 (1910), Arch. f. Entwickelungsmech., **31**, 658 (1910).

[2] Bioch. Zeitschr., **26**, 333 (1910).

[3] Bottazzi and Delfino, Centralbl. f. Physiol., **18**, 114; Savaré, Hofmeister's, Beiträge, **11**; Kikkoji, Zeitschr. f. physiol. Chem., **53**.

[4] Moscati, Zeitschr, f. physiol. Chem., **53**; Lochhead and Cramer, Proc. Roy. Soc., **80** B. (1908).

[5] Ascoli, Centralbl. f. Physiol., **16**; Raineri, Bioch. Centralbl., **4**, 428; Bergell and Liepmann, Münch. med. Wochenschr., 1905; Savaré, Hofmeister's Beiträge, **9**; Bergell and Falk, Münch. med. Wochenschr., **55**.

[6] See Etti, Maly's Jahresber., **2**, 287, and Preyer, Die Blutkristalle, Jena, 1871.

reaction, but quickly becomes acid. Its specific gravity is 1.033–1.040. It contains as form-elements fat-globules, small granules, and epithelium-cells. There have been found 81.2–120.9 p. m. solids, 61.2–105.6 p. m. protein, about 10 p. m. fat, and 3.7–8.2 p. m. ash in the uterine milk.

The fluid occurring in the so-called GRAPE-MOLE (MOLA racemosa) has a low specific gravity, 1.009–1.012, and contains 19.4–26.3 p. m. solids with 9–10 p. m. protein bodies and 6–7 p. m. ash.

The **amniotic fluid** in women is thin, whitish, or pale yellow; sometimes it is somewhat yellowish-brown and cloudy. White flakes separate. The form-elements are *mucus-corpuscles*, *epithelium-cells*, *fat-drops*, and *lanugo hair*. The odor is stale, the reaction neutral or faintly alkaline. The specific gravity is 1.002–1.028.

The amniotic fluid contains the constituents of ordinary transudates. The amount of solids at birth is scarcely 20 p. m. In the earlier stages of pregnancy the fluid contains more solids, especially proteins. Among the protein bodies, WEYL found one substance similar to *vitellin*, and with great probability also *seralbumin*, besides small quantities of *mucin*. Enzymes of various kinds (pepsin, diastase, thrombin, lipase) occur, according to BONDI. *Sugar* is regularly found in the amniotic fluid of cows, but not in human beings. In the ox, pig, and goat GÜRBER and GRÜNBAUM also found fructose. The human amniotic fluid also contains some *urea*, *uric acid*, *allantoin* and *creatinine* (AMBERG and ROWNTREE). The quantity of these may be increased in hydramnion (PROCHOWNICK, HARNACK), which depends on an increased secretion by the kidneys and skin of the fœtus. Lactates are doubtful constituents of the amniotic fluid. The quantity of urea in the amniotic fluid, is, according to PROCHOWNICK, 0.16 p. m. In the fluid in hydramnion PROCHOWNICK and HARNACK found, respectively, 0.34 and 0.48 p. m. urea. The principal mass of the solids consists of salts. The quantity of chlorides (NaCl) is 5.7–6.6 p. m. The molecular concentration of the amniotic fluid is somewhat lower than that of the blood, which is no doubt due to a dilution by the fœtal urine (ZANGEMEISTER and MEISSL [1]).

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[1] Weyl, Arch. f. (Anat. u.) Physiol., 1876; Bondi, Centralbl. f. Gynäkol., 1903; Prochownick, Arch. f. Gynäk., 11, also Maly's Jahresber., 7, 155; Harnack, Berlin. klin. Wochenschr., 1888, No. 41; Zangemeister and Meissl, Münch, med. Wochenschr. 1903; Gürber and Grünbaum, *ibid.*, 1904; Amberg and Rowntree, cited from Bioch. Centralbl., 10, 237.

CHAPTER XIII.

MILK.

THE chemical constituents of the *mammary glands* have been little studied. The cells are rich in protein and *nucleoproteins*. Among the latter we have one that yields pentose and guanine, on boiling with dilute mineral acids, but no other purine base. This *compound protein*, investigated by ODENIUS, contains as an average the following: 17.28 per cent N, 0.89 per cent S, and 0.277 per cent P. Besides this compound proteid we have at least one other, as MANDEL and LEVENE and LOEBISCH [1] have isolated a nucleic acid from the mammary gland, which, like the thymonucleic acids, yielded adenine, guanine, thymine, and cytosine. This nucleic acid also gave the pentose reactions and yielded an abundance of levulinic acid. Besides this nucleic acid, MANDEL and LEVENE isolated from the glands a glucothionic acid with 2.65 per cent S and 4.38 per cent N. Among the cleavage products of the nucleoprotein MANDEL [2] obtained no glycocoll, and the products of hydrolysis show a great correspondence with those of casein. We cannot state what relation the above-mentioned nucleic acids and the glucothionic acid bear to the not well-known constituent of the glands found by BERT and by THIERFELDER and which yields a reducing substance when boiled with dilute acids.

It is to be expected that these bodies are steps in the formation of milk-sugar; still we have no point of support for such an assumption, and recent investigations seem to indicate that the milk-sugar is produced in the glands by a transformation of the sugar of the blood. *Fat* seems, at least in the secreting glands, to be a never-failing constituent of the cells, and this fat may be observed in the protoplasm as large or small globules similar to milk-globules. The extractive bodies of the mammary glands have been little investigated, but among them are found considerable amounts of *purine bases*. The mammary glands also contain enzymes, among which we especially mention: catalase,

[1] Odenius, Maly's Jahresber., **30**; Mandel and Levene, Zeitschr. f. physiol. Chem., **46**; Loebisch, Hofmeister's Beiträge, 8.

[2] Mandel and Levene, Zeitschr. f. physiol. Chem., **45**, Mandel, Bioch. Zeitschr, **28**.

peroxidase and a proteolytic enzyme which, according to HILDEBRANDT,[1] occurs to a much greater extent in the active gland as compared with the inactive one.

As human milk and the milk of animals are essentially of the same constitution, it seems best to speak first of the one most thoroughly investigated, namely, cow's milk, and then of the essential properties of the remaining important kinds of milk.[2]

Cow's Milk.

Cow's milk, like every other kind, forms an emulsion which consists of very finely divided fat suspended in a solution consisting principally of protein bodies, milk-sugar, and salts. Milk is non-transparent, white, whitish-yellow, or in thin layers somewhat bluish-white, of a faint, insipid odor and mild, faintly sweetish taste. The specific gravity is 1.028 to 1.0345 at 15° C. The freezing-point is −0.54–0.59° C., average −0.563° C., and the molecular concentration 0.298.

The reaction of perfectly fresh milk is generally amphoteric toward litmus. The extent of the acid and alkaline part of this amphoteric reaction has been determined by different investigators, especially THÖRNER, SEBELIEN, and COURANT.[3] The results differ with the indicators used, and moreover the milk from different animals, as well as that from the same animal at different times during the lactation period, varies slightly. COURANT determined the alkaline part by N/10 sulphuric acid, using blue lacmoid as indicator, and the acid part by N/10 caustic soda, using phenolphthalein as indicator. He found, as an average for the first and last portions of the milking of twenty cows, that 100 cc. milk had the same alkaline reaction toward blue lacmoid as 41 cc. N/10 caustic soda, and the same acid reaction toward phenolphthalein as 19.5 cc. N/10 sulphuric acid. The actual reaction of cow's milk, which follows from the electrometric estimation, is, on the contrary, FOA[4] claims, nearly neutral, like the reaction of animal fluids and tissues in general.

Milk gradually changes when exposed to the air, and its reaction becomes more and more acid. This depends on a gradual transformation of the milk-sugar into lactic acid, caused by micro-organisms.

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[1] Bert, Compt. Rend., 98; Thierfelder, Pflüger's Arch., 34, and Maly's Jahresber., 13; Hildebrandt, Hofmeister's Beiträge, 5.

[2] A very complete reference to the literature on milk may be found in Raudnitz's "Die Bestandteile der Milch," in Ergebnisse der Physiol., 2, Abt. 1. The literature of the last few years may be found in the references by Raudnitz, Monatsschrift f. Kinderheilkunde.

[3] Thörner, Maly's Jahresber., 22; Sebelien, *ibid.*, Courant; Pflüger's Arch., 50.

[4] Compt. rend. soc. biolog. (58), 59, 51.

Perfectly fresh amphoteric milk does not coagulate on boiling, but forms a pellicle consisting of coagulated casein and lime-salts, which rapidly re-forms after being removed. After a sufficiently strong spontaneous formation of acid it coagulates on boiling, and lastly, when the formation of lactic acid is sufficient, it coagulates spontaneously at the ordinary temperature, forming a solid mass. It may also happen, especially in the warmth, that the casein-clot contracts and a yellowish or yellowish-green acid liquid (acid whey) separates.

Milk may undergo various fermentations. Lactic-acid fermentation, brought about by HÜPPE'S lactic-acid bacillus and also other varieties, takes first place. In the spontaneous souring of milk we generally consider the formation of lactic acid as the most essential product, but a formation of succinic acid may also take place, and in certain bacterial decompositions of milk, succinic acid and no lactic acid is formed. The materials from which these two acids are formed are lactose and lactophosphocarnic acid. Besides the lactic acids, the optically inactive as well as the dextro and levo acids, and succinic acid, volatile fatty acids, such as acetic acid, butyric acid, and others, may be formed in the bacterial decomposition of milk.

Milk sometimes undergoes a peculiar kind of coagulation, being converted into a thick, ropy, slimy mass (thick milk). This conversion depends upon a peculiar change in which the milk-sugar is made to undergo a slimy transformation. This transformation, which requires further investigation, is caused by special micro-organisms.

If the milk is sterilized by heating, and contact with micro-organisms prevented, the formation of lactic acid may be entirely stopped. The production of acid may also be prevented, at least for sometime, by many antiseptics, such as salicylic acid, thymol, boric acid, and other bodies.

If freshly drawn amphoteric milk is treated with rennet, it coagulates quickly, especially at the temperature of the body, to a solid mass (curd) from which a yellowish fluid (sweet whey) is gradually pressed out. This coagulation occurs without any change in the reaction of the milk, and therefore it is distinct from the acid coagulation.

In cow's milk we find as form-elements a few colostrum corpuscles (see Colostrum) and a few pale nucleated cells. The number of these form-elements is very small compared with the immense amount of the most essential form-constituents, the milk-globules.

The Milk-globules. These consist of extremely small drops of fat whose number is, according to WOLL,[1] 1.06–5.75 millions in 1 c.mm., and whose diameter is 0.0024–0.0046 mm. and 0.0037 mm. as an average for different kinds of animals. It is unquestionable that the milk-globules contain fat, and we consider it as positive that all the milk-fat exists in them. Another disputed question is whether the milk-globules consist entirely of fat or whether they also contain protein.

[1] On the Conditions Influencing the Number and Size of Fat-globules in Cow's Milk, Wisconsin Exp. Station, 6, 1892.

The observations of ASCHERSON[1] show that drops of fat, when dropped in an alkaline protein solution, are covered with a fine albuminous coat, a so-called *haptogen-membrane*. As milk on shaking with ether does not give up its fat, or only very slowly in the presence of a great excess of ether, and as this takes place very readily after the addition of acids or alkalies, which dissolve proteins, it was formerly thought that the fat-globules of the milk were enveloped in a protein coat. A true membrane has not been detected; and since, when no means of dissolving the protein is resorted to—for example, when the milk is precipitated by carbon dioxide after the addition of very little acetic acid, or when it is coagulated by rennet—the fat can be very easily extracted by ether, the theory of a special albuminous membrane for the fat-globule has been generally abandoned. The observations of QUINCKE[2] on the behavior of the fat-globules in an emulsion prepared with gum have led, at the present time, to the conclusion that each fat-globule in the milk is surrounded by a stratum of casein solution held by molecular attraction, and this prevents the globules from uniting with each other. Everything that changes the physical condition of the casein in the milk or precipitates it must necessarily help the solution of the fat in ether, and it is in this way that the alkalies, acids, and rennet act.

V. STORCH has shown, in opposition to these views, that the milk-globules are surrounded by a membrane of a special slimy substance. This substance is very insoluble, contains 14.2–14.79 per cent nitrogen, and yields a sugar, or at least a reducing substance, on boiling with hydrochloric acid. It is neither casein nor lactalbumin, but it seems to all appearances to be identical with the so-called "stroma substance" detected by RADENHAUSEN and DANILEWSKY. STORCH was able to show, by staining the fat-globules with certain dyes, that this substance enveloped them like a membrane. Recently VÖLTZ has given further proofs of the view that the fat-globules probably have a membrane, which in his opinion is a very labile formation of variable composition, and BAUER has also given further proofs for the assumption of a membrane. DROOP-RICHMOND and BONNEMA,[3] on the other hand, present several deductions conflicting with STORCH's theory. If STORCH's observation that the purified fat-globules contain a special protein substance differing from the dissolved proteins of the milk is correct, then the assumption as to a special body forming a membrane or stroma of the fat-globules becomes very probable. The correctness of STORCH's view has been substantiated very recently by ABDERHALDEN and VÖLTZ.[4] On the acid hydrolysis of the fat-globules they obtained glycocoll, which is absent in the casein as well as in the lactalbumin, and this shows that the

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[1] Arch. f. Anat. u. Physiol., 1840.

[2] Pflüger's Arch., 19.

[3] V. Storch, see Maly's Jahresber., 27; Radenhausen and Danilewsky, Forschungen auf dem Gebiete der Viehhaltung (Bremen, 1880), Heft 9; Völtz, Pflüger's Arch., 102; Bauer, Bioch. Zeitschr. 32; Droop-Richmond, see Chem. Centralbl., 1094, 2, 356; Bonnema, *ibid.*, 1243.

[4] Zeitschr. f. physiol. Chem., 59.

fat-globules at least cannot contain these two proteins alone. They must contain another protein, and it is still a question whether besides this they also contain casein and lactalbumin.

The milk-fat which is obtained under the name of butter consists mainly of *olein* and *palmitin*. Besides these it contains, as triglycerides, *myristic acid*, *stearic acid*, small amounts of *lauric acid*, *arachidic acid*, and *dioxystearic acid*, besides *butyric acid* and *caproic acid*, traces of *caprylic acid* and *capric acid*. RIEGEL claims that triglycerides of volatile fatty acids do not occur, but rather mixed triglycerides of volatile and non-volatile fatty acids. Milk-fat also contains small quantities of *phosphatides* (*lecithin*), and *cholesterin* and a yellow *coloring-matter*. The quantity of volatile fatty acids in butter is, according to DUCLAUX, on an average about 70 p.m., of which 37–51 p.m. is butyric acid and 30–33 p. m. is caproic acid. The non-volatile fat consists of $\frac{6}{10}$–$\frac{4}{10}$ olein and the remainder is principally palmitin. The composition of butter is not constant, but varies considerably under different circumstances.[1] The question whether the small fat-globules have a different composition from the large ones is still disputed.

The **milk-plasma**, or that fluid in which the fat-globules are suspended, contains several different proteins, the statements as to the number and nature of which are somewhat at variance. The three following, *casein*, *lactalbumin*, and *lactoglobulin*, have been most closely studied and are well characterized. The milk-plasma contains at least two carbohydrates, of which the one, *lactose*, is of great importance. It also contains extractive bodies, traces of *urea*, *creatine*, *creatinine*, *orotic acid*, *hypoxanthine* (?), *cholesterin*, *citric acid* (SOXHLET and HENKEL[2]), and lastly also *mineral bodies* and *gases*.

Casein. This protein substance, which thus far has been detected positively only in milk, belongs to the nucleoalbumins, and differs from the albuminates chiefly by its content of phosphorus and by its behavior, with the rennet enzyme. Casein from cow's milk has about the following composition: C 53.0, H 7.0, N 15.7, S 0.8, P 0.85, and O 22.65 per cent. Its specific rotation is, according to HOPPE-SEYLER, rather variable; in neutral solution it is $(\alpha)_D = -80°$; its faintly alkaline solution has a stronger rotation, namely, -97.8 to $-111.8°$, in a solution of N/10–N/5

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[1] Riegel, Maly's Jahresber., **34**; Duclaux, Compt. Rend., **104**. Various statement. as to the composition of milk-fat can be found in Koefoed, Bull. d. l'Acad. Roys Danoise, 1891, and Wanklyn, Chemical News, **63**; Browne, Chem. Centralbl., 1899, **2**, 883. In regard to the elementary composition of milk-fat see Fleischmann and Warmbold, Zeitschr., f. Biol., **50**.

[2] Cited from Söldner, Die Salze der Milch, etc., Landwirthsch. Versuchsstation, **35**, Separatabzug, 18.

NaOH (LONG [1]). The question whether the casein from different kinds of milk is identical or whether there are several caseins cannot be decided by the elementary analysis. According to TANGL and CSOKÁS, [2] mare's and ass's casein seem to be somewhat richer in nitrogen (16.44 and 16.28 per cent, respectively) but poorer in sulphur (0.528 and 0.588 per cent) and carbon (52.36 and 52.27 per cent) than the casein from cud chewers. The ass's casein was richer in phosphorus (1.057 per cent) than the mare's or cow's casein (both with 0.887 per cent).

Casein when dry appears like a fine white powder, which has no measurable solubility in pure water (LAQUEUR and SACKUR). Casein is only very slightly soluble in the ordinary neutral-salt solutions. According to ARTHUS it dissolves rather easily in a 1-per cent solution of sodium fluoride, ammonium or potassium oxalate. ROBERTSON thinks that it is more soluble in potassium cyanide and the alkali salts of certain volatile fatty acids such as butyric acid and valeric acid, than in solutions of the ordinary neutral salts. It is at least a tetrabasic acid, whose equivalent weight is 1135, according to LAQUEUR and SACKUR, and 1250 according to ROBERTSON. The statements as to the molecular weight are disputed (LAQUEUR and SACKUR, L. and D. VAN SLYKE [3]).

It dissolves readily in water with the aid of alkali or alkaline earths, also calcium carbonate, from which it expels carbon dioxide and it thus forms caseinates of variable composition. If casein is dissolved in lime-water and the solution carefully treated with very dilute phosphoric acid until it is neutral in reaction (to litmus), the casein appears to remain in solution, but is probably only swollen as in milk, and the liquid contains at the same time a large quantity of calcium phosphate without any precipitate or any suspended particles being visible. The casein solutions containing lime are opalescent, and have on warming the appearance of milk deficient in fat (which is also true for the salts of casein with the alkaline earths). Therefore it is not impossible that the white color of the milk is due partly to the casein and calcium phosphate. SÖLDNER and others have prepared two calcium compounds of casein with 1.5 p. c. CaO (the neutral caseinate according to SÖLDNER) and 2.4 p. c. CaO (the basic caseinate). The first is neutral to litmus while the other is neutral to phenolphthalein.

According to ROBERTSON [4] the alkali equivalent of casein at neutrality toward litmus $= 53 \times 10^{-5}$ equivalent-grm.-mol. per gram and at neutrality toward

[1] Hoppe-Seyler, Handb. d. physiol. u. pathol. chem. Analyse, 8. Aufl., 489; Long, Journ. Amer. Chem. Soc., **27**.

[2] Pflüger's Arch., **121**.

[3] Laqueur and Sackur, Hofmeister's Beiträge, **8**; M. Arthus, Thèses presentées à la faculté des sciences de Paris, 1893; Robertson, Journ. of biol. Chem., **2**; L. and D. van Slyke, Amer. Chem. Journ., **38**.

[4] See Ergebnis. d. Physiol. **10** and Journ. of physical Chem., **13**.

phenolphthalein $=80\times10^{-5}$ equivalent-grm.-mol. per gram. On saturation (with monacidic bases) the alkali equivalent is $=11\times10^{-5}$ grm.-mol. per gram. On saturating (with monobasic acids) the acid equivalent is $=32\times10^{-5}$ grm.-mol. per gram.

Besides the rather earlier investigations on the salts of casein by SÖLDNER, COURANT, RÖHMANN, LAQUEUR, RAUDNITZ [1] and others we have the recent observations and theoretical discussion of ROBERTSON [2] on the composition, nature and dissociation of the caseinates. We can here only refer to this and the earlier investigations.

Casein solutions do not coagulate on boiling, but solutions of casein-lime are covered, like milk, with a pellicle. They are precipitated by very little acid, but the presence of neutral salts retards the precipitation. A casein solution containing salt or ordinary milk requires, therefore, more acid for precipitation than a salt-free solution of casein of the same concentration. The precipitated casein dissolves very easily again in a small excess of hydrochloric acid, but less readily in an excess of acetic acid. The combination between casein and acid, like other protein and acid compounds, is precipitated by neutral salts. These acid solutions are precipitated by mineral acids in excess.[3] Casein is precipitated from neutral solutions or from milk by common salt containing calcium, or magnesium sulphate in substance, without changing its properties.[4] Metallic salts, such as alum, zinc sulphate and copper sulphate, completely precipitate the casein from neutral solutions.

On drying at 100° C., casein, according to LAQUEUR and SACKUR, decomposes and splits into two bodies. One of these, called *caseid*, is insoluble in dilute alkalies, while the other, the *isocasein*, is soluble therein. The isocasein is a stronger acid and has other precipitation limits and a rather lower equivalent weight than the casein.

The property which is the most characteristic of casein is that it coagulates with rennet in the presence of a sufficiently large amount of lime-salts. In solutions free from lime-salts the casein does not coagulate with rennet, but it is changed so that the solution (even if the enzymes are destroyed by heating) yields a coagulated mass, having the properties of a curd, if lime-salts are added. The rennet enzyme, rennin, has there

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[1] Söldner, Die Salze der Milch, etc., and Maly's Jahresber., **25**; Courant, l. c.; Röhmann, Berlin. klin. Wochenschr., 1895; Laqueur, l. c.; and Hofmeister's Beiträge, **7**; Raudnitz, Ergebn. d. Physiol., **2**, Abt. 1.

[2] Journ. of physical Chem., **11** and **12**; Journ. of biol. Chem., **5**.

[3] In regard to the acid combinations of casein and the ability to take up acid, see Laxa, Milchwirthsch. Centralbl., 1905; Long, Journ. Amer. Chem. Soc., **29**; L. and D. van Slyke, Amer. Chem. Journ., **28**; Robertson, Journ. of biol. Chem., **4**.

[4] See the works of Hammarsten and Schmidt-Nielsen, Hammarsten's Festschrift, 1906.

fore an action on casein even in the absence of lime-salts. These last are only necesary for the coagulation or the separation of the curd, and the process of coagulation is hence a two-phase process. The first phase is the transformation of the casein by the rennin, the second is the visible coagulation caused by the lime-salts. This fact, which was first proved by HAMMARSTEN, was later confirmed by ARTHUS and PAGÈS and recently closely studied by FULD, SPIRO, and LAQUEUR and others.[1]

The curd formed on the coagulation of milk contains large quantities of calcium phosphate. According to SOXHLET and SÖLDNER, the soluble lime-salts are of essential importance only in coagulation, while the calcium phosphate is without importance. COURANT believes that the calcium-casein on coagulation may carry down with it, if the solution contains dicalcium phosphate, a part of this as tricalcium phosphate, leaving mono-calcium phosphate in the solution. A solution of calcium casein is not coagulated by rennin alone but only when soluble lime-salts are added. Contrary to the generally accepted view that the soluble lime-salts are of importance in the coagulation, VAN DAM [2] claims that it is the quantity of lime combined with the casein which is of importance in the coagulation process. The rôle of the lime-salts in coagulation is not clear, and this follows from the chemical procedure in rennin coagulation.

If one makes use of a pure solution of casein and as pure rennin as possible, then after coagulation it is always found that the filtrate contains very small amounts of a protein, the *whey protein*, which is probably formed in the coagulation. This behavior, which was first shown by HAMMARSTEN, has been substantiated by many others and recently by FULD, SPIRO and SCHMIDT-NIELSEN. Whey protein is generally considered as a proteose substance, and KÖSTER [3] found 13.2 per cent nitrogen therein. In correspondence with these observations casein coagulation with rennin is considered as a cleavage process, in which the principal mass of the casein, sometimes more than 90 per cent, is split off as *paracasein*,[4] a body closely related to casein, and in the presence of sufficient

[1] See Maly's Jahresber., 2 and 4; also Hammarsten, Zur Kenntniss des Kaseïns und der Wirkung des Labfermentes, Nova Acta Reg. Soc. Scient. Upsala, 1877, Festschrift; Zeitschr. f. physiol. Chem., 22; Arthus et Pagès, Arch. de Physiol. (5), 2, and Mém. soc. biol., 43; Fuld, Hofmeister's Beiträge, 2, and Ergebnisse der Physiol., 1, Abt. 1, where a good review of the literature may be found, Spiro, Hofmeister's Beiträge, 6 and 7, with Reichel, *ibid.*, 7 and 8; Laqueur, *ibid.*, 7.

[2] Zeitschr. f. physiol. Chem., 58.

[3] Hammarsten, l. c.; Fuld. Bioch. Zeitschr., 4, and Hofmeister's Beiträge, 10; Spiro, Hofmeister's Beiträge, 8; Schmidt-Nielsen, Hammarsten's Festschrift, 1906; Köster, see Maly's Jahresber., 11, 14.

[4] It has been proposed to designate the ordinary casein as caseinogen and the curd as casein. Although such a proposition is theoretically correct, it leads in practice to confusion. On this account the author calls the curd paracasein, according to Schulze and Rõse (Landwirthsch. Versuchsstat., 31). A summary of the literature on the casein coagulation may be found in E. Fuld, Ergebnisse der Physiol., 1; Raudnits, *ibid.*, 2; and Laqueur, Biochem. Centralbl., 4, 344.

amounts of lime-salts the paracasein-lime precipitates out while the proteose-like substance (whey protein) remains in solution. In the coagulation in an acid medium the conditions are entirely different and proteoses and peptones are hereby formed to a considerable extent.

The paracasein is very similar to casein, but cannot be recoagulated by rennin. A solution of alkali-paracaseinate is much more readily precipitated by $CaCl_2$ than an alkali-caseinate solution of the same concentration, and the precipitation limits for saturated ammonium-sulphate solution, the upper as well as the lower limit, lie, according to LAQUEUR, lower with paracasein than with casein. The internal friction of paracasein solutions is also, in his opinion, less than that of casein solutions and indeed even to 20 per cent.

By continued action of rennin upon paracasein a further transformation has been found in many cases (PETRY, SLOWTZOFF, v. HERWERDEN [1]). This is explained by the presence of another proteolytic enzyme in the (impure) rennin preparation. This assumption seems to be plausible, and we are here probably dealing only with a secondary process which has nothing whatever to do with the true formation of paracasein. Whey protein is also formed after the very short action of rennin, and the continued cleavage occurs with varying speed. Thus SCHMIDT-NIELSEN found that the quantity of whey protein was even 3 per cent of the casein nitrogen after the action of rennet for 15 minutes, and only 4.25 per cent after 6 hours' action. These and other recent investigations favor the assumption that the casein coagulation by rennet is a hydrolytic cleavage, but the conditions are not so clear that this can be considered as proved.[2]

Fresh, unchanged milk does not, as is known, coagulate on boiling; but in not too rapid action of rennin a state may be observed in which the milk coagulates on heating (metacasein reaction). A solution of paracasein lactate, according to LAXA,[3] coagulates with rennin the same as a solution of casein lactate, which indicates, he believes, that the paracasein is transformed into casein again by the lactic acid. But as a precipitation of the paracasein from the acid solution is perhaps a pepsin action, the transformation of the paracasein into casein by the lactic acid must not be considered as proved.

In the digestion of casein with pepsin-hydrochloric acid primarily a phosphorized proteose is formed, from which then the pseudonuclein is split off (SALKOWSKI). The quantity thus split off is variable, as shown by the researches of SALKOWSKI, HAHN, MORACZEWSKI, SEBELIEN, and ZAITSCHEK.[4] The amount of phosphorus in the pseudonucleins obtained also varies considerably. SALKOWSKI considers that the quantity of pseudonuclein split off is dependent upon the relation between the casein and the digestion fluid, e.g., the quantity of the pseudonu-

[1] Petry, Hofmeister's Beiträge, 8; Slowtzoff, *ibid.*, 9; v. Herwerden, Zeitschr. f. physiol. Chem., 52; W. van Dam, *ibid.*, 61.

[2] See also Wernecken, Zeitschr., f. Biol., 52.

[3] Laxa, l. c.

[4] Salkowski, Zeitschr. f. physiol. Chem., 27; Salkowski and Hahn, Pflüger's Arch., 59; Salkowski, *ibid.*, 63; v. Moraczewski, Zeitschr. f. physiol. Chem., 20; Sebelien *ibid.*, 20; Zaitschek, Pflüger's Arch., 104.

cleins diminishes as the pepsin-hydrochloric acid increases. In the presence of 500 grams of pepsin-hydrochloric acid to 1 gram of casein, SALKOWSKI digested the latter completely without obtaining any pseudonuclein.

In peptic as well as tryptic digestion a part of the organic phosphorus is split off as orthophosphoric acid, the quantity increasing as the digestion progresses. Another part of the phosphorus is retained in organic combination in the proteoses as well as in the true peptones (SALKOWSKI, BIFFI, ALEXANDER, ADERS-PLIMMER and BAYLISS [1]).

From the products of peptic digestion of casein, after the separation of the pseudonuclein, SALKOWSKI [2] has isolated an acid rich in phosphorus. He considers this a *paranucleic acid.* This acid which gives the biuret test and a faint xanthoproteic reaction, contains 4.05–4.31 per cent phosphorus. A still richer product in phosphorus, with 6.9 per cent P, has been isolated by REH from the peptic digestive products of casein. He calls this body *polypeptid phosphoric acid.* This product, which also gives the above-mentioned protein reactions, and is not comparable with the nucleic acids, is characterized by a remarkably high content of amino-nitrogen, namely, 23.8 per cent. Among the products obtained by REH, DIETRICH [3] found a mixture of at least four different lime-salts of a peptone character, and which he considers as polypeptide-like combination with P_2O_5, *caseonphosphoric acids.* The amount of phosphorus was, respectively, 10.0, 4.1, 3.84 and 3.88 per cent.

Casein may be prepared in the following way: The milk is diluted with 4 vols. of water and the mixture treated with acetic acid to 0.75–1 p. m. Casein thus obtained is purified by repeatedly dissolving in water with the aid of the smallest quantity of alkali possible, by filtering and reprecipitating with acetic acid and thoroughly washing with water. Most of the milk-fat is retained by the filter on the first filtration, and the casein contaminated with traces of fat is purified by treating with alcohol and ether.

Lactoglobulin was obtained by SEBELIEN from cow's milk by saturating it with NaCl in substance (which precipitated the casein) and saturating the filtrate with magnesium sulphate. As far as it has been investigated it had the properties of serglobulin; the globulin isolated by TIEMANN [4] from colostrum had, nevertheless, a markedly low content of carbon, namely, 49.83 per cent.

Lactalbumin was first prepared in a pure state from milk by SEBELIEN. He gives its composition as, C 52.19, H 7.18, N 15.77, S 1.73, O 23.13 per cent. Lactalbumin has the properties of the albumins, and WICH-

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[1] Salkowski, l. c.; Biffi, Virchow's Arch., **152**; Alexander, Zeitschr. f. physiol. Chem., **25**; Plimmer and Bayliss, Journ. of Physiol., **33**; See also Küttner, Pflüger's Arch. **129**.

[2] Zeitschr. f. physiol. Chem., **32**.

[3] Reh. Hofmeister's Beiträge **11**; Dietrich, Bioch. Zeitschr. **22**.

[4] Zeitschr. f. physiol. Chem., **25**.

MANN found that it crystallizes in forms similar to ser- or ovalbumin. It coagulates, depending on the concentration and the amount of salt in solution, at 72–84° C. It is similar to seralbumin, but differs from it in having a considerably lower specific rotatory power: $(\alpha)_D = -37°$. According to FASAL[1] it is especially rich in tryptophane, namely, 3.07 per cent.

The principle of the preparation of lactalbumin is the same as for the preparation of seralbumin from serum. The casein and the globulin are removed by $MgSO_4$ in substance, and the filtrate treated as previously stated (page 263).

The occurrence of other proteins, such as *proteoses* and *peptones*, in milk has not been positively proved. These bodies are easily produced as laboratory products from the other proteins of the milk. Such a laboratory product is MILLON'S and COMAILLE'S *lactoprotein*, which is a mixture of a little casein with changed albumin, and proteose[2] which is formed by chemical action. In regard to *opalisin*, see Human Milk, p. 662.

Milk also contains, SIEGFRIED[3] claims, a *nucleon* related to phosphocarnic acid, which yields fermentation lactic acid (instead of paralactic acid) and a special carnic acid, *orylic acid* (instead of muscle carnic acid), as cleavage products. Lactophosphocarnic acid may be precipitated as an iron compound from the milk freed from casein and coagulable proteins as well as from earthy phosphates.

Milk also contains *enzymes* of various kinds. Of these we must mention *catalases*, *peroxidases*, and *reductases*, but the statements as to their occurrence in the milk from different animals as well as the question how much of their action is due to micro-organisms are conflicting. Among these enzyme actions a special interest has been given to the SCHARDINGER reaction, which consists in the fact that milk at 70° C. in the presence of formaldehyde or acetaldehyde reduces certain dyes, such as methylene blue, to leucobases. An *amylolytic enzyme* which converts starch into maltose occurs, especially, in human milk, while it is absent in cow's milk or occurs only to a slight extent. A *fermentation enzyme* which in the absence of micro-organisms decomposes the lactose into lactic acid, alcohol, and CO_2, occurs, according to STOKLASA[4] and his co-workers, in cow's milk as well as in human milk. Human milk, as well as cow's milk, contains a *lipase* which has the property at least of acting upon monobutyrin. BABCOCK and RUSSEL have found in these two kinds of milk, as well as certain others, a proteolytic

[1] Sebelien, Zeitschr., f. physiol. Chem., **9**; Wichmann, *ibid.*, **27**; Fasal, Bioch. Zeitschr., **44**.

[2] See Hammarsten, Maly's Jahresber., **6**, 13.

[3] Zeitschr. f. physiol. Chem., **21** and **22**.

[4] See Chem. Centralbl., 1905, **1**, 107.

enzyme which they call *galactase*, which is allied to trypsin, but differs therefrom in that it develops ammonia from milk even in the early stages of digestion. The occurrence of such an enzyme is denied by ZAITSCHEK and v. SZONTAGH, but on the other hand VANDEVELDE, DE WAELE, and SUGG [1] confirm the occurrence of a proteolytic enzyme in milk.

Orotic acid, $C_5H_{11}N_2O_4.2H_2O$, is the name given by BISCARO and BELLONI [2] to a new constituent of milk which they have discovered. This acid, which can be precipitated by basic lead acetate from whey free from protein, is slightly soluble in water, crystalline, and gives several crystalline salts. The monomethyl and ethyl esters of this acid are also known. It yields urea on treatment with potassium permanganate.

Lactose, MILK-SUGAR, $C_{12}H_{22}O_{11}+H_2O$. This sugar, on hydrolysis, can be split into two hexoses, *glucose* and *galactose*. It yields mucic acid besides other organic acids, by the action of dilute nitric acid. Levulinic acid is formed, besides formic acid and humin substances, by the stronger action of acids. By the action of alkalies, among other products we find lactic acid and pyrocatechin.

Milk-sugar occurs, as a rule, only in milk, but it has also been found in the urine of pregnant women, on stagnation of milk, as well as in the urine after partaking of large quantities of the same sugar.

Lactose occurs ordinarily as colorless rhombic crystals with 1 molecule of water of crystallization, which is driven off by slowly heating to 100° C., but more easily at 130–140° C. On quickly boiling down a milk-sugar solution, anhydrous milk-sugar separates out. Milk-sugar dissolves in 6 parts cold or in 2.5 parts boiling water; it has a faintly sweetish taste. It does not dissolve in ether or absolute alcohol. Its solutions are dextrogyrate. The rotatory power, which on heating the solution to 100° C. becomes constant, is $(\alpha)_D = +52.5°$. Milk-sugar combines with bases; the alkali combinations are insoluble in alcohol.

Milk-sugar is not fermentable with pure yeast. It undergoes, on the contrary, alcoholic fermentation by the action of certain schizomycetes, and E. FISCHER [3] found that the milk-sugar is first split into glucose and galactose by an enzyme, *lactase*, existing in the yeast. The preparation of milk-wine, "*kumyss*," from mare's milk and "*kephir*" and "*yoghurt*" from cow's milk is based upon this fact. Other micro-organisms also take part in this change, causing a lactic-acid fermentation of the milk-sugar.

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[1] Babcock and Russel, Centralbl. f. Bakt. u. Parisitenkunde (II), **6**, and Maly's Jahresber., **31**; Zaitschek and v. Szontagh, Pflüger's Arch., **104**; Vandevelde, de Waele, and Sugg, Hofmeister's Beiträge, **5**.

[2] See Chem. Centralbl., 1905, **2**, 63.

[3] Ber. d. d. Chem. Gesellsch., **27**.

Lactose responds to the reactions of glucose, such as MOORE'S,[1] TROMMER'S and RUBNER'S, and the bismuth test. It also reduces mercuric oxide in alkaline solutions. After warming with phenylhydrazine acetate it gives on cooling a yellow crystalline precipitate of phenyl lactosazone, $C_{24}H_{32}N_4O_9$. It differs from cane-sugar by giving positive reactions with MOORE'S or TROMMER'S and the bismuth test, and also in that it does not darken when heated to 100° C. with anhydrous oxalic acid. It differs from glucose and maltose by its solubility and crystalline form, but especially, by its not fermenting with yeast, and by yielding mucic acid with nitric acid.

The osazone obtained with phenylhydrazine acetate, which melts at 200° C., differs from the other osazones by being inactive when 0.2 gram is dissolved in 4 cc. of pyridine and 6 cc. of absolute alcohol and viewed through a layer 10 centimeters long (NEUBERG[2]).

For the preparation of milk-sugar we make use of the by-product in the preparation of cheese, the sweet whey. The protein is removed by coagulation with heat, and the filtrate evaporated to a syrup. The crystals which separate after a certain time are recrystallized from water after decolorizing with animal charcoal. A pure preparation may be obtained from the commercial milk-sugar by repeated recrystallization. The quantitative estimation of milk-sugar may be performed either by the polaristrobometer or by means of titration with FEHLING'S solution. Ten cc. of FEHLING'S solution are reduced by 0.0676 gram of milk-sugar in 0.5–1.5 per cent solution after boiling for six minutes. (In regard to FEHLING'S solution and the titration of sugar see larger hand-books.)

From the non-correspondence between the quantity of sugar in the milk as determined by polarization and gravimetrically, when the polarization results are always higher, SEBELIEN[3] has concluded that the milk must contain a second reducing substance which polarizes stronger than lactose. This substance is probably a pentose and occurs to a very slight extent in ordinary milk, 0.25–0.35 p. m. (SEBELIEN and SUNDE), and more in colostrum, 0.5 p. m.

RITTHAUSEN found another carbohydrate in milk which is soluble in water, non-crystallizable, which has a faint reducing action, and which yields, on boiling with an acid, a body having a greater reducing power. BÉCHAMP[4] considers this as dextrin.

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[1] The well-known beautiful red color, which milk produces after the addition of alkali, at the room temperature and to which attention has been called recently by Gautier, Morel, and Monod (Compt. rend. soc. biol., **60** and **62**), and Krüger (Zeitschr. f. Physiol. Chem., **50**) is a Moore's reaction modified by the presence of protein and perhaps also other milk constituents.

[2] Ber. d. d. Chem. Gesellsch., **32**.

[3] Sebelien, Hammarsten's Festschrift, 1906; with Sunde, Zeitschr. f. angew. Chem., **21**.

[4] Ritthausen, Journ. f. prakt. Chem. (N. F.), **15**; Béchamp, Bull. Soc. Chim. (3), **6**.

The *mineral bodies* of milk will be treated in connection with its quantitative composition.

The methods for the quantitative analysis of milk are very numerous, and as all cannot be treated here, we will give the principal points of a few of the methods considered most trustworthy and most frequently employed.

In determining the *solids* a carefully weighed quantity of milk is mixed with an equal weight of heated quartz sand, fine glass powder, or asbestos. The evaporation is first done on the water-bath and finished in a current of carbon dioxide or hydrogen not above 100° C.

The *mineral bodies* are determined by incinerating the milk, using the precautions mentioned in the text-books. The results obtained for the phosphoric acid are incorrect on account of the burning of phosphorized bodies, such as casein and lecithin. We must, therefore, according to SÖLDNER, subtract in round numbers 25 per cent from the total phosphoric acid found in the milk. The quantity of sulphate in the ash also depends on the combustion of the proteins.

In the determination of the *total amount of proteins* RITTHAUSEN'S method is employed, namely, the precipitation of the milk with copper sulphate according to the modification suggested by MUNK.[1] He precipitates all the proteins by means of cupric hydroxide at boiling heat, and determines the nitrogen in the precipitate by means of KJELDAHL'S method. This modification gives more exact results.

According to SEBELIEN'S method, three to four grams of milk are diluted with an equal volume of water, a little common-salt solution added, and the proteins precipitated with an excess of tannic acid. The precipitate is washed with cold water, and then the quantity of nitrogen determined by KJELDAHL'S method. The total nitrogen found when multiplied by 6.37 (casein and lactalbumin contain both 15.7 per cent nitrogen) gives the total quantity of proteins. This method, which is readily performed, gives very good results. I. MUNK used this method in the analysis of woman's milk. In this case the quantity of nitrogen found must be multiplied by 6.34. G. SIMON[2] found that the precipitation with tannic acid, also with phosphotungstic acid, is the simplest and most accurate. The objection to this and other methods in which the proteins are precipitated is that perhaps other bodies (extractives) may be carried down at the same time (CAMERER and SÖLDNER[3]). It is not known to what extent this takes place.

A part of the nitrogen in the milk exists as extractives, and this nitrogen is calculated as the difference between the total nitrogen and the protein nitrogen. According to MUNK'S analyses about $\frac{1}{7}$ of the total nitrogen belongs to the extractives in cow's milk. CAMERER and SÖLDNER determine the nitrogen in the filtrate from the tannic-acid precipitate by KJELDAHL'S method, and also according to HÜFNER'S method (hypobromite). In this way they found 18 milligrams of nitrogen according to HÜFNER (urea, etc.) in 100 grams of cow's milk.

To determine the *casein* and *albumin* separately we may make use of the method first suggested by HOPPE-SEYLER and TOLMATSCHEFF,[4] in which the casein is precipitated by magnesium sulphate. According to SEBELIEN the milk is diluted with its own volume of a saturated magnesium-sulphate solution, then saturated with the salt in substance, and the precipitate then filtered and washed with a saturated magnesium-sulphate solution. The nitrogen is determined in the pre-

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[1] Ritthausen, Journ. f. prakt. Chem. (N. F.), 15; I. Munk, Virchow's Arch., 134.

[2] Sebelien, Zeitschr. f. physiol, Chem., 13; Simon, *ibid.*, 33.

[3] Zeitschr. f. Biologie, 33 and 36.

[4] Hoppe-Seyler, Med. chem. Untersuch., 272.

cipitate by KJELDAHL'S method, and the quantity of casein (+globulin) determined by multiplying the result by 6.37. The quantity of lactalbumin may be calculated as the difference between the casein and the total proteins found. The lactalbumin may also be precipitated by tannic acid from the filtrate from the casein precipitate containing $MgSO_4$, after diluting with water, the nitrogen determined by KJELDAHL'S method and the result multiplied by 6.37.

SCHLOSSMANN [1] suggests an alum solution, which precipitates the casein, in order to separate the casein from the other proteins, and the albumin is then precipitated from the filtrate by tannic acid. The nitrogen in the precipitate is determined by the KJELDAHL method. This method has recently been tested by SIMON and he recommends it highly.

The *fat* is gravimetrically determined by thoroughly extracting the dried milk with ether, evaporating the ether from the extract, and weighing the residue. The fat may be determined by aerometric means by adding alkali to the milk, shaking with ether, and determining the specific gravity of the fat solution by means of SOXHLET'S apparatus. In determining the amount of fat in a large number of samples the lactocrit of DE LAVAL may be used with success. There are numerous other methods for estimating milk-fat, but they cannot be considered here.

In determining the *milk-sugar* the proteins are first removed. For this purpose we precipitate either with alcohol, which must be evaporated from the filtrate, or by diluting with water, and removing the casein by the addition of a little acid, and the lactalbumin by coagulation at boiling heat. The sugar is determined by titration with FEHLING'S or KNAPP'S solution (see Chapter XIV). The principle of the titration is the same as for the titration of sugar in the urine; 10 cc. of FEHLING'S solution correspond to 0.0676 gram of milk-sugar; 10 cc. of KNAPP'S solution correspond to 0.0311–0.0310 gram of milk-sugar, when the saccharine liquid contains about ½–1 per cent of sugar. In regard to the *modus operandi* of the titration we must refer the reader to more extensive works.

Instead of these volumetric determinations other methods of estimation, such as ALLIHN'S method, the polariscope method, and others, may be used. In calculating the analysis or in determining the solids it is of importance to remember, as suggested by CAMERER and SÖLDNER, that the milk-sugar in the residue is anhydrous. Many other methods for determining the milk-sugar have been suggested and recommended.

The *quantitative composition* of cow's milk is naturally very variable. The average obtained by KÖNIG [2] is as follows in 1000 parts:

Water.	Solids.	Casein.	Albumin.	Fats.	Sugar.	Salts.
871.7	128.3	30.2	5.3	36.9	48.8	7.1
		35.5				

The quantity of *mineral bodies* in 1000 parts of cow's milk is, according to the analyses of SÖLDNER, as follows: K_2O 1.72, Na_2O 0.51, CaO 1.98, MgO 0.20, P_2O_5 1.82 (after correction for the pseudonuclein), Cl 0.98 grams. BUNGE found 0.0035 gram Fe_2O_3, and EDELSTEIN and CSONKA [3] found 0.0007–0.001 gm. Fe_2O_3. According to SÖLDNER the K, Na, and Cl are found in the same quantities in whole milk as in milk-serum. Of the total phosphoric acid 36–56 per cent, and of the lime

[1] Zeitschr. f. physiol. Chem., 22.

[2] Chemie der menschlichen Nahrungs- und Genussmittel, 4. Aufl.

[3] Bunge, Zeitschr. f. Biol. 10; Edelstein and Csonka, Bioch. Zeitschr., 38.

53–72 per cent is not in simple solution. A part of this lime is combined with the casein; the remainder is found united with the phosphoric acid as a mixture of dicalcium and tricalcium phosphates which is kept dissolved or suspended by the casein. RONA and MICHAELIS [1] found that about 40–50 per cent of the total quantity of lime was diffusable; according to them nearly one-half of the calcium is contained in the milk as a non-dissociable casein compound, while the milk only contains the very smallest amounts of suspended calcium phosphate.

The bases are in excess of the mineral acids in the milk-serum. The excess of the first is combined with organic acids, which correspond to 2.5 p. m. citric acid (SÖLDNER).

The *gases* of the milk consist mainly of CO_2, besides a little N and traces of O. PFLÜGER [2] found 10 vols. per cent CO_2 and 0.6 vol. per cent N calculated at 0° C. and 760 mm. pressure.

The variation in the composition of cow's milk depends on several circumstances.

The **colostrum**, or the milk which is secreted before calving and in the first few days after, is yellowish, sometimes alkaline, but often acid, of higher specific gravity, 1.046–1.080, and richer in solids than ordinary milk. The colostrum contains, besides fat-globules, an abundance of colostrum-corpuscles—nucleated granular cells 0.005–0.025 mm. in diameter with abundant fat-granules and fat-globules. The fat of colostrum has a somewhat higher melting-point and is poorer in volatile fatty acids than the fat from ordinary milk (NILSON [3]). The iodine equivalent of the colostrum-fat is higher than that of milk-fat. The quantity of cholesterin and lecithin is generally greater. The most apparent difference between it and ordinary milk is that colostrum coagulates on heating to boiling because of the absolutely and relatively greater quantities of globulin and albumin that it contains.[4] The composition of colostrum varies considerably. KÖNIG gives as average the following figures in 1000 parts:

Water.	Solids.	Casein.	Albumin and Globulin.	Fat.	Sugar.	Salts.
746.7	253.3	40.4	136.0	35.9	26.7	15.6

The influence which food exercises upon the composition of milk will be discussed in connection with the chemistry of the milk secretion.

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[1] Bioch. Zeitschr., **21**.

[2] Pflüger's Arch., **2**.

[3] See Maly's Jahresber., **21**. See also Engel and Bode, Zeitschr. f. physiol. Chem., **74**.

[4] See Sebelien, Maly's Jahresber., 18, and Tiemann, Zeitschr. f. physiol. Chem., **25**. See also Simon, *ibid.*, **33**; Winterstein and Strickler, *ibid.*, **47**.

In the following table is given the average composition of skimmed milk and certain other preparations of milk:

	Water.	Proteins.	Fat.	Sugar.	Lactic Acid.	Salts.
Skimmed milk.....	906.6	31.1	7.4	47.5	...	7.4
Cream.....	655.1	36.1	267.5	35.2	...	6.1
Buttermilk.....	902.7	40.6	9.3	37.3	3.4	6.7
Whey.....	932.4	8.5	2.3	47.0	3.3	6.5

KUMYSS, KEPHIR and YOGHURT are obtained, as above stated, by the alcoholic and lactic-acid fermentation of the milk-sugar, the first from mare's milk and the other from cow's milk. Large quantities of carbon dioxide are formed thereby, and besides this the protein bodies of the milk are partly converted into proteoses and peptones, which increase the digestibility. The quantity of lactic acid in these preparations may be about 10–20 p. m. The quantity of alcohol varies from 10 to 35 p. m.

Milk of Other Animals. GOAT'S milk has a more yellowish color and a more specific odor than cow's milk. The coagulum obtained by acid or rennet is more solid and is harder than that from cow's milk. SHEEP'S milk is similar to goat's milk, but has a higher specific gravity and contains a greater amount of solids.

MARE'S milk is alkaline and contains a casein which is not precipitated, by acids, in lumps or solid masses, but, like the casein from woman's milk, in fine flakes. This casein is only incompletely precipitated by rennet, and it is very similar also in other respects to the casein of human milk. In BEIL'S [1] opinion the casein from mare's and cow's milk is the same, and the different behavior of the two varieties of milk is due to varying amounts of salts and to a different relation between the casein and the albumin. This does not agree with the analyses of casein by TANGL and CSOÁKS given above nor with the investigations of ZAITSCHEK and V. SZONTAGH, who find that the casein from mare's milk, like that from human and ass's milk, is digested by pepsin-hydrochloric acid without leaving a residue. According to ENGEL and DENNEMARK [2] the colostrum from the mare differs from that from the ass by being richer in casein than the milk. The milk of the ASS is claimed by earlier authorities to be similar to human milk' but SCHLOSSMANN finds it considerably poorer in fat. The researches of ELLENBERGER give similar results, and show great similarity between ass's milk and human milk. The average results were 15 p. m. protein with 5.3 p. m. albumin and 9.4 p. m. casein. This latter, like human casein, does not yield any pseudonuclein on pepsin digestion, which agrees well with the above-mentioned investigations of ZAITSCHEK. The quantity of nucleon was about the same as in woman's milk. The quantity of fat was 15 p. m., and the sugar was 50–60 p. m. REINDEER milk is characterized, according to WERENSKIOLD,[3] by being very rich in fat, 144.6–197.3 p. m., and casein, 80.6– 86.9 p. m.

The milk of CARNIVORA (the bitch and cat) is acid in reaction and very rich in solids. The composition of the milk of these animals varies with the composition of the food.

To illustrate the composition of the milk of other animals the following figures, the compilation of KÖNIG, are given. As the milk of each kind of animal may have a variable composition, these figures should only be considered as examples of the composition of milk of various kinds:[4]

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[1] Studein über die Eiweissstoffe des Kumys und Kefirs, St. Petersburg, 1886 (Ricker).

[2] Zeitschr. f. physiol. Chem., **76**.

[3] Zaitschek, l. c.; Schlossmann, Zeitschr. f. physiol. Chem., **22**; Ellenberger, Arch. f. (Anat. u.) Physiol., 1899 and 1902; Werenskiold, Maly's Jahresber., **25**.

[4] Details in regard to the milk of different animals may be found in Pröscher, Zeitschr. f. physiol. Chem., **24**; Abderhalden, *ibid.*, **27**. In regard to pig milk, see Zunts and Ostertag, Landw. Jahresb., **37**.

Milk of the	Water.	Solids	Proteins.	Fat.	Sugar.	Salts.
Dog	754.4	245.6	99.1	95.7	31.9	7.3
Cat	816.3	183.7	90.8	33.3	49.1	5.8
Goat	869.1	130.9	36.9	40.9	44.5	8.6
Sheep	835.0	165.0	57.4	61.4	39.6	6.6
Cow	871.7	128.3	35.5	36.9	48.8	7.1
Horse	900.6	99.4	18.9	10.9	66.5	3.1
Ass	900.0	100.0	21.0	13.0	63.0	3.0
Pig	823.7	176.3	60.9	64.4	40.4	10.6
Elephant	678.5	321.5	30.9	195.7	88.5	6.5
Dolphin	486.7	513.3	....	437.6	....	4.6
Whale [1]	698.0	302.0	94.3	194.0		9.9

Human Milk.

Woman's milk is amphoteric in reaction. According to COURANT its reaction is relatively more alkaline than cow's milk, but it has, nevertheless, a lower absolute reaction for alkalinity as well as for acidity. He found between the tenth day and the fourteenth month after confinement practically constant results. The alkalinity, as well as the acidity, was a little lower than in childbed. One hundred cc. of the milk had the same average alkalinity as 10.8 cc. N/10 caustic soda, and the same acidity as 3.6 cc. N/10 acid. The relation between the alkalinity and the acidity in woman's milk was as 3:1, and in cow's milk as 2.1:1. The actual reaction determined electrometrically is, according to FOÀ,[2] still nearly neutral, like the other kinds of milk. ALLARIA has also arrived at similar results, according to whom the tendency of human milk toward alkaline reaction even in the most prominent cases never corresponds to a $\frac{N}{1{,}000{,}000}$NaOH solution.

Human milk also contains fewer fat-globules than cow's milk, but they are larger in size. The specific gravity of woman's milk varies between 1.026 and 1.036, generally between 1.028 and 1.034. It is highest in well-fed and lowest in poorly-fed women. The freezing-point is lowered on an average 0.589° C., according to WINTER and PARMENTIER[3] constant at 0.55°, and the molecular concentration is 0.318.

The fat of woman's milk has been investigated by RUPPEL. It forms a yellowish-white mass, similar to ordinary butter, having a specific gravity of 0.966 at 15°. It melts at 34.0° C. and solidifies at 20.2° C. The following fatty acids can be obtained from the fat, namely, butyric, caproic, capric, myristic, palmitic, stearic, and oleic acids. The fat from woman's milk is, according to RUPPEL and LAVES,[4] relatively poor in volatile fatty acids. The non-volatile fatty acids consist of one-half oleic acid,

[1] Scheibe, cited in Bioch. Centralbl., **7**, 553.

[2] Compt. rend. soc. biol. **58**; Allaria, Maly's Jahresb., **29**, 242.

[3] See Maly's Jahresber., **24**.

[4] Ruppel, Zeitschr. f. Biologie, **31**; Laves, Zeitschr. f. physiol. Chem., **19**.

while among the solid fatty acids myristic and palmitic acids are found to a greater extent than stearic acid.

The essential qualitative difference between woman's and cow's milk seems to lie in the proteins or in the more accurately determined *casein*. A number of both the earlier and more recent investigators[1] claim that the casein from woman's milk has other properties than that from cow's milk. The essential differences are the following: The casein from woman's milk is precipitated with greater difficulty with acids or salts. It does not coagulate uniformly in the milk after the addition of rennet, which depends, essentially, upon the low amount of lime-salts and casein contained in the milk.[2] It may be precipitated by gastric juice, but dissolves completely and easily in an excess of gastric juice; the casein precipitate produced by an acid is more easily soluble in an excess of the acid; and lastly, the clot formed from the casein of woman's milk does not appear in such large and coarse masses as in the casein from cow's milk, but is more loose and flocculent. This last-mentioned fact is of great importance, since it explains the generally admitted fact of the easy digestibility of the casein from woman's milk.

The question as to whether the above-mentioned variations depend on a decided difference in the two caseins, or only on an unequal relation between the casein and the salts in the two kinds of milk, or upon other circumstances, has not as yet been decided. According to SZONTAGH and ZAITSCHEK and also WRÓBLEWSKY, the casein from human milk does not yield any pseudonuclein on peptic digestion, and hence it cannot be a nucleoalbumin. According to KOBRAK, woman's casein yields some pseudonuclein, and with repeated solution in alkali and precipitation by an acid it becomes more and more like cow's casein. He therefore suggests the possibility that woman's casein is a compound between a nucleoalbumin and a basic protein. WRÓBLEWSKY found the following for the composition of casein from woman's milk: C 52.24, H 7.32, N 14.97, P 0.68, S 1.117 per cent. LANGSTEIN and BERGELL obtained much lower figures for N, S and especially P, namely, 14.34, 0.85 and 0.27 per cent, respectively. According to LANGSTEIN and EDELSTEIN the phosphorus content is only 0.22–0.29 per cent. On hydrolysis ABDERHALDEN and LANGSTEIN[3] could not find any difference between cow and human casein.

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[1] See Biedert, Untersuchungen über die chemischen Unterschiede der Menschen- und Kuhmilch (Stuttgart), 1884; Langgaard, Virchow's Arch., 64; Makris, Studien über die Eiweisskörper der Frauen- und Kuhmilch, Inaug.-Diss. Strassburg, 1876.

[2] See among others Bienenfeld, Bioch. Zeitschr., 7, and Fuld and Wohlgemuth, *ibid.*, 8.

[3] Szontagh, Maly's Jahresber., 22; Zaitschek, l. c.; Wróblewsky, Beiträge zur Kenntniss des Frauenkaseins, Inaug.-Diss. Bern. 1894, and Ein neuer eiweissartiger

Woman's milk also contains lactalbumin, besides the casein, and a protein substance, very rich in sulphur (4.7 per cent) and relatively poor in carbon, which WROBLEWSKY calls *opalisin*. The statements as to the occurrence of proteoses and peptones are conflicting as in many other cases. No positive proof as to the occurrence of proteoses and peptones in fresh milk has been given.

Because of the properties and low amount of casein in human milk it is often difficult to precipitate it, with acid, and to prepare it, but this can easily be accomplished by dialysis. A number of methods have been suggested for the preparation of human casein. FULD and WOHLGEMUTH recommend the freezing of the milk previous to precipitation, so that the casein masses become larger to a certain extent and the precipitation becomes easier. ENGEL[1] recommends dilution with water to 5 volumes, and the addition of 60–80 cc. N/10 acetic acid for each 100 cc. milk. The mixture is first cooled for 2–3 hours and then, after shaking, warmed on the water-bath to 40° for a few minutes.

Even after those differences are eliminated which depend on the imperfect analytical methods employed, the *quantitative composition of woman's milk* is variable to such an extent that it is impossible to give any average results. The numerous analyses, especially those made on a large number of samples by PFEIFFER, ADRIANCE, CAMERER and SÖLDNER,[2] have positively shown that woman's milk is essentially poorer in proteins but richer in sugar than cow's milk. The quantity of protein varies between 10–20 p. m., often amounting to only 15–17 p. m. or less, and is dependent upon the length of lactation (see below). The quantity of fat also varies considerably, but ordinarily amounts to 30–40 p. m. The quantity of sugar should not be below 50 p. m., but may rise to even 80 p. m. About 60 p. m. may be considered as an average, but it should be borne in mind that the quantity of sugar is also dependent upon the length of lactation, as it increases with duration. The amount of mineral bodies varies between 2 and 4 p. m.

The division of the total nitrogen in human milk is, according to A. FREHN,[3] very variable. As approximate average figures we can say that 40–45 per cent of the total nitrogen is casein, 35–40 per cent remain-

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Bestandteil der Milch, Anzeiger der Akad. d. Wiss. in Krakau, 1898; Kobrak, Pflüger's Arch., 80; Langstein and Bergell, cited in Bioch. Centralbl., 8, 323; Langstein and Edelstein, Maly's Jahresber, 40, 254; Abderhalden and Langstein. Zeitschr., f. physiol. Chem.. 66.

[1] Fuld and Wohlgemuth, Bioch. Zeitschr., 5; Engel, *ibid.*, 14.

[2] Pfeiffer, Jahrb. f. Kinderheilkunde, 20, also Maly's Jahresber., 13; V. Adriance and J. Adriance, A Clinical Report of the Chemical Examination, etc., Archives of Pediatrics, 1897; Camerer and Söldner, Zeitschr. f. Biologie, 33 and 36. In regard to the composition of Woman's milk, see also Biel, Maly's Jahresber., 4; Christenn, *ibid.*, 7; Mendes de Leon, *ibid.*, 12; Gerber, Bull. soc. chim., 23; Tolmatscheff, Hoppe-Seyler's Med.-chem. Untersuch., 272.

[3] Zeitschr. f. physiol. Chem., 65; see also Engel and Frehn, Maly's Jahresber., 40.

ing proteins and about 20 per cent for rest nitrogen. The principal part of the rest nitrogen is considered as urea.

From a quantitative standpoint, the most essential differences between woman's and cow's milk are the following: As compared with the quantity of albumin, the quantity of casein is not only absolutely but also relatively smaller in woman's milk than in cow's milk, while the latter is poorer in milk-sugar. Human milk is richer in lecithin, at least relatively to the amount of protein. BUROW found 0.49–0.58 p. m. lecithin in cow's milk and 0.58 p. m. in woman's milk, which corresponds to 1.40 per cent for the first milk and 3.05 per cent for the second, calculated on the percentage of protein. NERKING and HAENSEL found as average for lecithin in cow's milk 0.63 p. m. and in woman's milk 0.50 p. m. GLIKIN found 0.765 p. m. lecithin (phosphatides) as average for cow's milk and 1.329 p. m. for human milk. KOCH found that both human milk and cow's milk contain lecithin as well as cephalin. The total quantity of both bodies in human milk was 0.78 p. m. and in cow's milk 0.72–0.86 p. m. The quantity of nucleon is greater in woman's milk. WITTMAACK claims that cow's milk contains 0.566 p. m. nucleon, and woman's milk 1.24 p. m., and according to VALENTI the quantity of nucleon in human milk is indeed still higher. SIEGFRIED finds that the nucleon phosphorus amounts to 6.0 per cent of the total phosphorus in cow's milk and 41.5 per cent in woman's milk, and also that in human milk the phosphorus is almost all in organic combination. This does not agree with the results of SIKES who found on an average of only 42 per cent of the total P_2O_5 in organic combination. Because of the large amount of casein (and calcium phosphate) cow's milk is much richer in phosphorus than human milk. The relation P_2O_5:N, according to SCHLOSSMANN,[1] is equal to 1:5.4 in human milk and 1:2.7 in cow's milk. Woman's milk is poorer in mineral bodies, especially lime, and it contains only one-sixth of the quantity of lime as compared with cow's milk. The mineral constituents of human milk are better assimilated by the organism of the nursing child than those of cow's milk. Human milk is also claimed to be poorer in citric acid (SCHEIBE[2]), although this is not an essential difference.

Another difference between woman's milk and other varieties of milk is UMIKOFF'S reaction, which seems to depend upon the quantitative composition, especially the relation between the milk-sugar, citric acid, lime, and iron (SIEBER[3]). This reaction consists in treating 5 cc. of woman's milk with 2.5 cc. ammonia

[1] Burow, Zeitschr. f. physiol. Chem., **30**; Koch. *ibid.*, **47**; Wittmaack, *ibid.*, **22**; Siegfried, *ibid.*, **22**; Nerking and Haensel, Bioch. Zeitschr., **18**; Glikin, *ibid.*, **21**; Valenti, Biochem. Centralbl., **4**; Schlossmann, Arch. f. Kinderheilkunde, **40**; Sikes, Journ. of Physiol., **34**.

[2] Maly's Jahresber., **21**.

[3] Zeitschr. f. physiol. Chem., **30**.

(10 per cent) and heating to 60° C. for 15–20 minutes, when the mixture becomes violet-red. Cow's milk gives a yellowish-brown color when thus treated.

According to RUBNER woman's milk contains about 3 p. m. soaps, but this could not be substantiated by CAMERER and SÖLDNER. They conclude that woman's milk contains no soaps, or at least only very small amounts. They also found the quantity of urea nitrogen in woman's milk to be 0.11–0.12 p. m., although SCHÖNDORFF [1] found nearly twice this amount, namely, 0.23 p. m.

In regard to the quantity of *mineral bodies* in woman's milk we have the analyses of several investigators, especially of BUNGE (analyses *A* and *B*) and of SÖLDNER and CAMERER (analysis *C*).[2] BUNGE analyzed the milk of a woman, fourteen days after delivery, whose diet contained very little common salt for four days previous to the analysis (*A*), and again three days later after a daily addition of 30 grams of NaCl to the food (*B*). The figures are in 1000 parts of the milk·

	A	*B*	*C*
K_2O	0.780	0.703	0.884
Na_2O	0.232	0.257	0.357
CaO	0.328	0.343	0.378
MgO	0.064	0.065	0.053
Fe_2O_3	0.004	0.006	0.002
P_2O_5	0.473	0.469	0.310
Cl	0.438	0.445	0.591

The relation of the two bodies potassium and sodium to each other may, BUNGE believes, vary considerably (1.3–4.4 equivalents of potash to 1 of soda). By the addition of salt to the food, the quantity of sodium and chlorine in the milk increases, while the quantity of potassium decreases. DE LANGE found more Na than K in the milk at the beginning of lactation. JOLLES and FRIEDJUNG found on an average 5.9 milligrams of iron per liter of woman's milk. CAMERER and SÖLDNER [3] find about the same amount, namely, 10–20 milligrams Fe_2O_3 = 3.5–7 milligrams iron in 1000 grams human milk.

The gases of woman's milk have been investigated by KÜLZ.[4] He found 1.07 –1.44 cc. of oxygen, 2.35–2.87 cc. of carbon dioxide, and 3.37–3.81 cc. of nitrogen in 100 cc. of milk.

The proper treatment of cow's milk by diluting it with water and by certain additions in order to render it a proper substitute for woman's milk in the nourishment of children cannot be determined before the difference in the protein bodies of these two kinds of milk has been completely studied.

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[1] Rubner, Zeitschr. f. Biologie, **36**; Camerer and Söldner, *ibid.*, **39**; Schöndorff, Pflüger's Arch., **81**.

[2] Bunge, Zeitschr. f. Biologie, **10**; Camerer and Söldner, *ibid.*, **39** and **44**.

[3] De Lange, Maly's Jahresber., **27**; Jolles and Friedjung, Arch. f. exp. Path. u. Pharm., **46**; Camerer and Söldner, Zeitschr. f. Biologie, **46**.

[4] Zeitschr. f. Biologie, **32**.

The **colostrum** has a higher specific gravity, 1.040–1.060, a greater quantity of coagulable proteins, and a deeper yellow color than ordinary woman's milk. Even a few days after delivery the color becomes less yellow, the quantity of albumin less, and the number of colostrum-corpuscles diminishes.

We have the older analyses of CLEMM [1] and the recent investigations of PFEIFFER, V. and J. ADRIANCE, CAMERER and SÖLDNER on the changes in the composition of milk after delivery. It follows, as a unanimous result from these investigations, that the quantity of protein, which amounts to more the first two days, sometimes to more than 30 p. m. at first, rather quickly and then more generally diminishes as long as the lactation continues, so that in the third week it equals about 10–18 p. m. Like the protein substances, the mineral bodies also gradually decrease. The quantity of fat shows no regular or constant variation during lactation, while the lactose, especially according to the observations of V. and J. ADRIANCE (120 analyses), increases rather quickly the first days and then only slowly until the end of lactation. The analyses of PFEIFFER, CAMERER and SÖLDNER also show an increase in the quantity of milk-sugar.

The two mammary glands of the same woman may yield somewhat different milk, as shown by SOURDAT and later by BRUNNER.[2] Likewise the different portions of milk from the same milking may have varying composition. The first portions are always poorer in fat.

According to L'HÉRITIER and to VERNOIS and BECQUEREL, the milk of blondes contains less casein than that of brunettes, a difference which TOLMATSCHEFF [3] could not substantiate. Women of delicate constitutions yield a milk richer in solids, especially in casein, than women with strong constitutions (V. and B.).

According to VERNOIS and BECQUEREL, the age of the woman has an effect on the composition of the milk, so that we find a greater quantity of proteins and fat in women 15–20 years old and a smaller quantity of sugar. The smallest quantity of proteins and the greatest quantity of sugar are found at 20 or from 25 to 30 years of age. VERNOIS and BECQUEREL, consider that the milk with the first-born is richer in water—with a proportionate diminution of casein, sugar, and fat—than after several deliveries.

The influence of menstruation seems to diminish slightly the milk-sugar and to increase considerably the fat and casein (VERNOIS and BECQUEREL).

Witch's milk is the secretion of the mammary glands of new-born children of both sexes immediately after birth. This secretion has from a qualitative standpoint the same constitution as milk, but may show important differences and variations from a quantitative point of view. SCHLOSSBERGER and HAUFF, GUBLER and QUEVENNE, and V. GENSER,[4] have made analyses of this milk and give the following results: 10.5–28 p. m. proteins, 8.2–14.6 p. m. fat, and 9–60 p. m. sugar.

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[1] See Hoppe-Seyler, Physiol. Chem., 734.

[2] Sourdat, Compt. Rend., **71**; Brunner, Pflüger's Arch., **7**.

[3] l'Héritier, cited from Hoppe-Seyler, Physiol. Chem., 738; Vernois and Becquerel, Du lait chez la femme dans l'état de santé, etc., (Paris, 1853); Tolmatscheff, Hoppe-Seyler, Med.-chem. Untersuch., 272.

[4] Schlossberger and Hauff, Annal. d. Chem., u. Pharm., **96**; Gubler and Quevenne, cited from Hoppe-Seyler's Physiol. Chem., 723; v. Genser, *ibid.*

As milk is the only form of nourishment during a certain period of the life of man and mammals, it must contain all the nutriment necessary for life. This fact is shown by the milk containing representatives of the three principal groups of organic nutritive substances—proteins, carbohydrates, and fat, and the last two groups can here also in part mutually substitute each other. Besides this all milk seems to contain, without doubt, some lecithin and nucleon. The mineral bodies in milk must also occur in proper proportions, and on this point the experiments of BUNGE on dogs are of special interest. He found that the mineral bodies of the milk occur in about the same relative proportion as they do in the body of the sucking animal. BUNGE[1] found in 1000 parts of the ash the following results (*A* represents results from the new-born dog, and *B* the milk from the bitch):

	A	*B*
K_2O	114.2	149.8
Na_2O	106.4	88.0
CaO	295.2	272.4
MgO	18.2	15.4
Fe_2O_3	7.2	1.2
P_2O_5	394.2	342.2
Cl	83.5	169.0

BUNGE explains the fact that the milk-ash is richer in potash and poorer in soda than the new-born animal by saying that in the growing animal the ash of the muscles rich in potash relatively increases and the cartilage rich in soda relatively decreases. In regard to the amount of iron we find an unexpected condition, the ash of the new-born animal containing six times as much as the milk-ash. This condition BUNGE explains by the fact founded on his and ZALESKY's experiments, that the quantity of iron in the entire organism is highest at birth. The new-born has therefore its own supply of iron for the growth of its organs even at birth.

The investigations of HUGOUNENQ, DE LANGE, CAMERER and SÖLDNER[2] have shown that in man the conditions are different from those in animals, as the ash of the child has an entirely different composition as compared with the milk. As an example the following analyses are given (of CAMERER and SÖLDNER). (*A*, the ash of the sucking infant, and *B*, the ash of the milk.) The results are in 1000 parts of the ash.

	A	*B*
K_2O	78	314
Na_2O	91	119
CaO	361	164
MgO	9	26
Fe_2O_3	8	6
P_2O_5	389	135
Cl	77	200

[1] Zeitschr. f. physiol. Chem., **13**.

[2] Hugounenq, Compt. Rend., **128**; de Lange, Zeitschr., f. Biologie, **40**; Camerer and Söldner, *ibid.*, **39**, **40**, and **44**.

We cannot therefore state as a definite fact that the composition of the ash of the sucking young and the ash of the corresponding milk coincide. BUNGE[1] nevertheless claims that the composition of the ash of the sucking young of various mammals is nearly the same, but that the ash of the milk differs from the ash of the young in so far as the slower the young grows the richer it is in alkali chlorides and relatively poorer in phosphates and lime-salts. The constituents of the ash have two functions to perform, namely, the building up of the tissues and secondly the preparation of the excreta, especially the urine. The faster the young grows the more is the first in evidence, while the slower it develops, the more prominent is the second.

The quantity of mineral bodies in the milk, and especially the amount of lime and phosphoric acid, as shown by BUNGE and PRÖSCHER and PAGÈS, stands in close relation to the rapidity of growth, because the amount of these mineral constituents in the milk is greater in animals which grow and develop quickly than in those which grow only slowly. A similar relation also exists, as shown by the researches of PRÖSCHER, and especially of ABDERHALDEN,[2] between the quantity of protein in the milk and the rapidity of development of the sucking young. The amount of protein is greater in the milk the quicker the animal develops.

The *influence of the food* on the composition of the milk is of interest from many points of view and has been the subject of many investigations. From these we learn that in human beings as well as in animals an insufficient diet decreases the quantity of milk and the quantity of solids, while abundant food increases both. From the observations of DECAISNE[3] on nursing women during the siege of Paris in 1871, the amount of casein, fat, sugar, and salts, but especially the fat, was found to decrease with insufficient food, while the quantity of lactalbumin was found to be somewhat increased. Food rich in proteins increases the quantity of milk, and also the solids contained, especially the fat, according to most reports. The quantity of sugar in woman's milk is found by certain investigators to be increased after food rich in proteins, while others claim it is diminished. A diet rich in fat may, as the researches of SOXHLET and many others[4] have shown, cause a marked increase in the fat of the milk when the fat partaken is in a readily digestible and assimilable form. The presence of large quantities of carbohydrates in the food

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[1] Bunge, " Die zunehmende Unfähigkeit der Frauen ihre Kinder zu stillen," München, 1900, cited by Camerer, Zeitschr. f. Biologie, 40.

[2] Pröscher, Zeitschr. f. physiol. Chem., 24; Abderhalden, *ibid.*, 27; Pagès, Arch. de Physiol. (5), 7.

[3] Cited from Hoppe-Seyler, l. c., 739.

[4] See Maly's Jahresber., 26. See also Basch, Ergebnisse der Physiologie, 2, Abt. 1.

seems to cause no constant, direct action on the quantity of the milk constituents.[1] From feeding experiments with different foods we come to the conclusion that the character of the food is of comparatively little influence, while the race and other conditions play an important rôle. Watery food gives a milk containing an excess of water and having little value. In the milk from cows which were fed on distillers' grain COMMAILLE[2] found 906.5 p..m. water, 26.4 p. m. casein, 4.3 p. m. albumin, 18.2 p. m. fat, and 33.8 p. m. sugar. Such milk has sometimes a peculiar sharp after-taste, although not always. TANGL and ZAITSCHEK[3] could not find any difference in the average composition of the milk produced after feeding with dry and with moist fodder.

Chemistry of Milk-secretion. That the constituents which occur actually dissolved in milk pass into the secretion and not alone by filtration or diffusion, but more likely are secreted by a specific secretory activity of the granular elements, is shown by the fact that milk-sugar, which is not found in the blood, is to all appearances formed in the glands themselves. A further proof lies in the fact that the lactalbumin is not identical with seralbumin; and lastly, as BUNGE[4] has shown, the mineral bodies secreted by the milk are in quite different proportions from those in the blood-serum.

Little is known in regard to the formation and secretion of the specific constituents of milk. The older theory, that the casein was produced from the lactalbumin by the action of an enzyme, is incorrect, and probably originated from mistaking an alkali albuminate for casein. Better founded is the theory that the casein originates from the protoplasm of the gland-cells. According to BASCH'S researches, the casein is formed in the mammary gland by the nucleic acid of the nucleus being set free and uniting intra-alveolar with the transudated serum, thus forming a nucleoalbumin, the casein. The untenableness of this view has been shown by LÖBISCH, and the investigations, of HILDEBRANDT[5] upon the proteolytic enzyme of the mammary gland, and the autolysis

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[1] In regard to the literature on the action of various foods on woman's milk, see Zalesky, "Ueber die Einwirkung der Nahrung auf die Zusammensetzung und Nahrhaftigkeit der Frauenmilch," Berlin. klin. Wochenschr., 1888, which also contains the literature on the importance of diet on the composition of other kinds of milk. In regard to the extensive literature on the influence of various foods on the milk production of animals, see König, Chem. d. menschl. Nahrungs und Genussmittel. 3. Aufl., 1, 298. See also Maly's Jahresber., 29–40, and Morgen, Beger and Fingerling, Landw. Versuchsst., 61, and Raudnitz, Monatschr. f. Kinderheilk.

[2] Cited from König, 2, 235.

[3] Landwirt. Vers. St. 1911.

[4] Lehrbuch d. physiol. und pathol. Chem., 3. Aufl., 93.

[5] Basch, Jahrb. f. Kinderheilkunde, 1898; Hildebrandt, Hofmeister's Beiträge, 5; Löbisch. *ibid.*, 8.

of the gland have not given any clue as to the mode of formation of casein. The findings of MANDEL [1] that the hydrolytic cleavage products of the nucleoprotein from the mammary glands occur approximately quantitatively in the same proportions as in casein, are important in this connection.

That the milk-fat is produced by a formation of fat in the protoplasm, and that the fat-globules are set free by their destruction, is a generally admitted opinion, which, however, does not exclude the possibility that the fat is in part taken up by the glands from the blood and eliminated with its secretion. That the fats of the food can pass into the milk follows from the investigations of WINTERNITZ, as he has been able to detect the passage of iodized fats in the milk, and these observations have been substantiated by the investigations of CASPARI and PARASCHTSCHUK.[2] The abundant quantities of iodized fat which were eliminated with the milk in these cases without doubt depend, at least in great part, upon the iodized fat of the food, hence it cannot be said that all of the milk-fat containing iodine was unchanged iodized fat of the food. The previously-mentioned older investigations of LEBEDEFF and ROSENFELD and also the recent ones of SPAMPANI and DADDI, PARASCHTSCHUK, GOGITIDSE and others on the passage of foreign fats into the milk also indicate the passage of the fat of the food into the milk, although we are still uncertain on this point. According to SOXHLET the fat of the food does not pass into the milk directly, but is destroyed in place of the body-fat, which then becomes available and is, as it were, pushed into the milk. HENRIQUES and HANSEN could not detect any mentionable quantity of linseed-oil in the milk after feeding with this oil; the milk-fat was not normal, but had a higher iodine equivalent and a higher melting-point, from which they also concluded that a transformation of the food-fat in the glandular cells is possible. The results of the experiments of GOGITIDSE [3] with soaps also indicate that the mammary glands have the property of forming fats by synthesis from their components. As a formation of fat from carbohydrates in the animal organism is at the present day considered as positively proved, it is likewise possible that the milk-glands also produce fats from the carbohydrates brought to them by the blood. It is a well-known fact that an animal gives off for a long time, daily, considerably more fat in the milk than it receives

[1] Bioch. Zeitschr., **22**.

[2] Winternitz, Zeitschr. f. physiol. Chem., **24**; Caspari, Arch. f. (Anat. u.) Physiol., 1899, Supplbd. and Zeitschr. f. Biologie, **46**; with Winternitz, *ibid.*, **49**; Paraschtschuk, Chem. Centralbl., 1903, **1**.

[3] Lebedeff, Pflüger's Arch. **31**; Rosenfeld, Ergebn. d. Physiol. **1** and **2**; Spampani and Daddi, Maly's Jahresber., **26**; Henriques and Hansen, *ibid.*, **29**; Gogitidse, Zeitschr. f. Biologie, **45**, **46**, and **47**. See also Basch, Ergebnisse d. Physiol., **2**, Abt. **1**.

as food, and this proves that at least a part of the fat secreted by the milk is produced from proteins or carbohydrates, or perhaps from both. The question as to how far this fat is produced directly in the milk-glands, or from other organs and tissues, and brought to the gland by means of the blood, cannot be decided.

The origin of milk-sugar is not known. MÜNTZ calls attention to the fact that a number of very widely diffused bodies in the vegetable kingdom—vegetable mucilage, gums, pectin bodies—yield galactose as a product of decomposition, and he believes, therefore, that milk-sugar may be formed in herbivora by a synthesis from glucose and galactose. This origin of milk-sugar does not apply to carnivora, as they produce milk-sugar when fed on food consisting entirely of lean meat. The observations of BERT and THIERFELDER [1] that a mother-substance of the milk-sugar, a saccharogen, occurs in the glands, does not explain the formation of milk-sugar, as the nature of this mother-substance is still unknown. As the animal body has undoubtedly the power of converting one variety of sugar into another, the origin of the milk-sugar can be sought simply in the glucose introduced as food or formed in the body. Certain observations of PORCHER indicate such an origin as he found in sheep, cows, and goats whose mammary glands were extirpated, that glucose appeared in the urine after delivery. He also found that milk secreting animals became glycosuric on the removal of the mammary glands, and he explains this glycosuria by the fact that the lactose-forming action of the gland was removed at the time of delivery, when large amounts of glucose were being produced. The experiments of KAUFMANN and MAGNE upon cows also indicate a formation of lactose from glucose. They found that during secretion the glands took sugar from the blood, so that the venous gland-blood was poorer in sugar than otherwise. NOEL-PATON and CATHCART [2] have carried on experiments on phlorhinized dogs which show a lactose formation from glucose.

The passage of foreign substances into the milk stands in close connection with the chemical processes of milk secretion.

It is a well-known fact that milk acquires a foreign taste from the food of the animal, which is in itself a proof that foreign bodies pass into the milk. This fact becomes of special importance in reference to such injurious substances as may be introduced into the organism of the nursing child by means of the milk.

Among these substances may be mentioned opium and morphine, which after large doses pass into the milk and act on the child. Alcohol

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[1] Müntz, Compt. Rend., **102**; Bert and Thierfelder, footnote 1, p. 644.

[2] Porcher, Compt. Rend. **138** and **141** and Bioch. Zeitschr., **23**; Kaufmann and Magne, Compt. Rend., **143**; Noel-Paton and Cathcart, Journ. of Physiol., **42**.

may also pass into the milk, but probably not in such quantities as to have any direct action on the nursing child.[1] Alcohol is claimed to have been detected in the milk after feeding cows with brewer's grains.

Among inorganic bodies, iodine, arsenic, bismuth, antimony, zinc, lead, mercury, and iron have been found in milk. In icterus neither bile-acids nor bile-pigments pass into the milk.

Under diseased conditions no constant change has been found in woman's milk. In isolated cases SCHLOSSBERGER, JOLY and FILHOL [2] have indeed observed a markedly abnormal composition, but no positive conclusion can be derived therefrom.

The changes in cow's milk in disease have been little studied. In tuberculosis of the udder, STORCH [3] found tubercle bacilli in the milk, and he also noted that the milk became more and more diluted, during the disease, with a serous liquid similar to blood-serum, so that that the glands finally, instead of yielding milk, gave only blood-serum or a serous fluid. HUSSON [4] found that milk from murrain cows contained more proteins but considerably less fat and (in severe cases) less sugar than normal milk.

The milk may be blue or red in color, due to the development of micro-organisms.

The formation of concrements in the exit-passages of the cow's udder is often observed. These consist chiefly of calcium carbonate, or of carbonate and phosphate with only a small amount of organic substances.

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[1] See Klingemann, Virchow's Arch., **126**, and Rosemann, Pflüger's Arch., **78**.

[2] Schlossberger, Annal. d. Chem. u. Pharm., **96**; Joly and Filhol, cited from v. Gorup-Besanez, Lehrb., 4, Aufl., 438.

[3] See Bang, Om Tuberkulose i Koens Yver og om tuberkulös Mälk, Nord. Med. Arkiv, **16**, and also Maly's Jahresber., **14**, 170; Storch, Maly's Jahresber., **14**.

[4] Compt. Rend., **78**.

CHAPTER XIV.

URINE.

URINE is the most important excretion of the animal organism; it is the means of eliminating the nitrogenous metabolic products, also the water and the soluble mineral substances; and in many cases it furnishes important data relative to the metabolism, quantitatively by its variation, and qualitatively by the appearance of foreign bodies in the excretion. Moreover, in many cases we are able, from the chemical or morphological constituents which the urine abstracts from the kidneys, ureter, bladder, and urethra, to judge of the condition of these organs; and lastly urinary analysis affords an excellent means of deciding the question as to how certain medicinal agents or other foreign substances introduced into the organism are absorbed and chemically changed. In this respect, urinary analysis has furnished very important particulars especially in regard to the nature of the chemical processes taking place within the organism, and it is therefore not only an important aid to the physician in diagnosis, but it is also of the greatest importance to the toxicologist and the physiological chemist.

In studying the secretions and excretions, the relation must be sought between the chemical structure of the secreting organ and the chemical composition of its secreted products. Investigations with respect to the kidneys and the urine have led to very few results from this standpoint. Although the anatomical relation of the kidneys has been carefully studied, their chemical composition has not been the subject of thorough analytical research. In cases in which a chemical investigation of the kidneys has been undertaken, it has been in general only of the organ as such, and not of the different anatomical parts. An enumeration of the chemical constituents of the kidneys known at the present time can, therefore, only haye a secondary value.

In the kidneys we find proteins of different kinds. According to HALLIBURTON the kidneys do not contain any albumin, but only a *globulin* and a *nucleoprotein*. The globulin coagulates at about 52° C., and the nucleoprotein contains 0.37 per cent phosphorus. LIEBERMANN claims that the kidneys contain a *lecithalbumin*, and he ascribes to this body a special importance in the secretion of acid urines. The

kidneys also contain, according to LÖNNBERG, a *mucin-like substance*. This substance yields no reducing body on boiling with acids, and belongs chiefly to the papillæ, and is, this author says, a nucleoalbumin (nucleoproteid?). The cortical substance is richer in another nucleoalbumin (nucleoproteid) unlike mucin. It has not been decided what relation this last substance bears to HALLIBURTON'S nucleoprotein. *Chondroitin sulphuric acid* also occurs as traces. MANDEL and LEVENE have also obtained *glucothionic acid* from the kidneys, and the question as to the relation of this to the renosulphuric acid described by MANDEL and NEUBERG [1] is still undecided. This renosulphuric acid to all appearances is not a unit substance but a sulphuric acid ester, and a component related to glucuronic acid which contained 2.63 p. c. S., 4.53 p. c., N., and 1.34 p. c. P.

Fat occurs only in very small amounts and this fat, like the organ fat in general, is relatively rich in unsaturated fatty acids. The *phosphatides* seem to be of different kinds. FRÄNKEL and NOGUEIRA [2] found a cephalin-like substance, a triaminodiphosphatide and a diaminomonophosphatide. DUNHAM and JACOBSON [3] found in beef-kidneys a substance which they called *carnaubon* which is soluble in alcohol but insoluble in ether, and which is a triaminomonophosphatide with the formula $C_{74}H_{150}N_3PO_{13}$. Carnaubon does not contain any glycerin but an amino-sugar, two choline groups and a molecule of each of the following acids: stearic, palmitic and carnaubic ($C_{24}H_{48}O_2$) acids. Among the extractive bodies of the kidneys one finds *purine bases*, *betaine*,[4] *urea*, *uric acid* (traces), *glycogen*, *leucine*, *inosite*, *taurine*, and *cystine* (in ox-kidneys). The quantitative analyses of the kidneys thus far made possess little interest. In the kidney of a healthy suicide MAGNUS-LEVY [5] found in 1000 parts of the fresh substance 756 p. m. water, 244 p. m. solids, 52.7 p. m. fat, 2.08 p. m. Cl., 0.192 p. m. Ca., 0.207 p. m. Mg and 0.158 p. m. Fe.

The fluid collected under pathological conditions, as in hydronephrosis, is thin with a variable but generally low specific gravity. Usually it is straw-yellow or paler in color, and sometimes colorless. Most frequently it is clear, or only faintly cloudy from white blood-corpuscles and epithelium-cells; in a few cases it is so rich in form-elements that it appears like pus. Protein generally occurs in small amounts; occasionally it is entirely absent, but in a few rare cases the

[1] Halliburton, Journ. of Physiol., **13**, Suppl., and **18**; Liebermann, Pflüger's Arch., **50** and **54**; Lönnberg, see Maly's Jahresber., **20**; Mandel and Levene, Zeithschr. f. physiol. Chem., **47**; Mandel and Neuberg, Bioch. Zeitschr., **18**; Mörner, Skand. Arch. f. Physiol., **6**.

[2] Bioch. Zeitschr., **16**.

[3] Zeitschr. f. physiol. Chem., **64**.

[4] Bebeschin, Zeitschr., f. physiol. Chem., **72**.

[5] Bioch. Zeitschr., **24**.

amount is nearly as large as in the blood-serum. Urea occurs sometimes in considerable amounts when the parenchyma of the kidneys is only in part atrophied; in complete atrophy the urea may be entirely absent.

I. PHYSICAL PROPERTIES OF URINE.

Consistency, Transparency, Odor, and Taste of Urine. Under physiological conditions urine is a thin liquid and gives, when shaken with air, a froth which quickly subsides. Human urine, or urine from carnivora, which is habitually acid, appears clear and transparent, often faintly fluorescent, immediately after voiding. When allowed to stand for a little while human urine shows a light cloud (*nubecula*), which consists of the so-called "*mucus*," and generally also contains a few epithelium cells, mucus-corpuscles, and urate-granules. The presence of a larger quantity of urates renders the urine cloudy, and a clay-yellow, yellowish-brown, rose-colored, or often brick-red precipitate (*sedimentum lateritium*) settles on cooling, because of the greater insolubility of the urates at the ordinary temperature than at the temperature of the body. This cloudiness disappears on gently warming. In new-born infants the cloudiness of the urine during the first 4–5 days is due to epithelium, mucus-corpuscles, uric acid, and urates. The urine of herbivora, which is habitually neutral or alkaline in reaction, is very cloudy on account of the carbonates of the alkaline earths present. Human urine may sometimes be alkaline under physiological conditions. In this case it is cloudy, due to the earthy phosphates, and this cloudiness does not disappear on warming, differing in this respect from the *sedimentum lateritium*. Urine has a salty and faintly bitter taste produced by sodium chloride and urea. The odor of urine is peculiarly aromatic; the bodies which produce this odor are unknown.

The **color** of urine is normally pale yellow when the specific gravity is 1.020. The color otherwise depends on the concentration of the urine and varies from pale straw-yellow, when the urine contains small amounts of solids, to a dark reddish-yellow or reddish-brown in stronger concentration. As a rule the intensity of the color corresponds to the concentration, but under pathological conditions, exceptions occur such as are found in diabetic urine, which contains a large amount of solids and has a high specific gravity and a pale-yellow color.

The **reaction** of urine depends essentially upon the composition of the food. The carnivora, as a rule, void an acid, the herbivora, a neutral or alkaline urine. If a carnivore is put upon a vegetable diet, its urine may become less acid or neutral, while the reverse occurs when an herbivore is starved, that is, when it lives upon its own tissues, as then the urine voided is acid.

The urine of a healthy man on a mixed diet has an *acid reaction*,

and the sum of the acid equivalents is greater than the sum of the basic equivalents. This depends upon the fact that in the physiological combustion of neutral substances (proteins and others) within the organism, acids are produced, chiefly sulphuric acid, but also phosphoric and organic acids, such as hippuric, uric, and oxalic acids, aromatic oxyacids, oxyproteic acids and others. From this it follows that the acid reaction is not due to one acid alone. The various acids take part in the acid reaction in proportion to their dissociation, since, according to the ion theory, the acid reaction of a mixture is dependent upon the number of hydrogen ions present. Hence the theory that the acidity is due entirely to dihydrogen phosphate is incorrect although this salt takes such a great part in the acid reaction that its quantity is often taken as a measure of the degree of acidity of the urine.[1]

The composition of the food is not the only influence which affects the degree of acidity of human urine. For example, after taking food at the beginning of digestion, when a larger amount of gastric juice containing hydrochloric acid is secreted, the urine may be neutral or even alkaline.[2] As to the time of the appearance of the maximum and minimum of acidity, the various investigators do not agree, which may in part be explained by the varying individuality and conditions of life of the persons investigated. It has not infrequently been observed that perfectly healthy persons in the morning void a neutral or alkaline urine which is cloudy from earthy phosphates. The effect of muscular activity on the acidity of urine has not been positively determined. According to HOFFMANN, RINGSTEDT, ODDI, and TARULLI and VOZÁRIK muscular work raises the degree of acidity, but ADUCCO [3] claims that it decreases it. Abundant perspiration reduces the acidity (HOFFMANN).

In man and especially in carnivora it seems that the degree of acidity of the urine cannot be increased above a certain point, even though mineral acids or organic acids which are burned up with difficulty are ingested in large quantities. Under such conditions a different behavior has been repeatedly observed between carnivora and herbivora. In the first (and also in man) it has been found that the acids are in part neutralized by the alkalies and alkaline earths of the body, but that the excess of acid is combined with ammonia, split off from the proteins or their cleavage products, and eliminated in the urine as ammonium salt. In herbivora such a combination of the excess of acid with ammonia

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[1] In regard to the acidity of the urine see the recent works of Ringer, Zeitschr. f. physiol. Chem. **60**; Henderson, Bioch. Zeitschr. **24**, with Spiro, *ibid.*, **15**; De Jager, Maly's Jahresb. **39** and Bioch. Zeitschr. **38**; v. Skramlik, Zeitschr. f. physiol. Chem. **71**; Klein and Moritz, Deutsch. Arch. f. klin. Med. **99**; Quagliariello, Chem. Centralbl. 1912, 1, 506.

[2] Contradictory statements are found in Linossier, Maly's Jahresber., **27**.

[3] Hoffmann, see Maly's Jahresber., **14**; Ringstedt, *ibid.*, **20**; Oddi and Tarulli, *ibid.*, **24**; Aducco, *ibid.*, **17**; Vozárik, Pflüger's Arch., **111**.

seems not to take place, or not to the same extent,[1] and this is given as a reason why herbivora soon die when acids are given. This is true at least for rabbits, while according to BAER this power of increasing the elimination of ammonia exists also in the goat, monkey, and pig, hence no definite difference in this regard exists between herbivora and carnivora. The differences which have been observed are, according to EPPINGER, not of a special kind, and they may be caused, he says, from a different amount of protein in the food which yields ammonia. Thus dogs with food poor in protein behave like rabbits while, according to EPPINGER, in herbivora (rabbits) a de-toxification of the acid can be brought about by the abundant supply of proteins or their cleavage products. The correctness of this statement is still disputed (POHL) or has only been partly confirmed (BOSTOCK). The point is disputed and it must not be forgotten that, as A. LOEWY[2] found, the sensitiveness toward the action of acids varies very much in different individuals.

Although one cannot raise the degree of acidity of the urine above a certain limit by the introduction of acid, still it may be easily diminished, so that the reaction becomes neutral or alkaline. This occurs after the taking of carbonates of the fixed alkalies or of such alkali salts of vegetable acids—citric acid, and malic acid—as are easily burned into carbonates in the organism. Under pathological conditions, as in the absorption of alkaline transudates, or the alkaline fermentation within the bladder, the urine may become alkaline.

A urine with an alkaline reaction caused by fixed alkalies has a very different diagnostic value from one whose alkaline reaction is caused by the presence of ammonium carbonate. In the latter case we have to deal with a decomposition of the urea of the urine by the action of micro-organisms.

If one wishes to determine whether the alkaline reaction of the urine is due to ammonia or to fixed alkalies, a piece of red litmus paper is dipped into the urine and allowed to dry exposed to the air or to a gentle heat. If the alkaline reaction is due to ammonia, the paper becomes red again; but if it is caused by fixed alkalies, it remains blue.

Determination of the Acidity. As the quantity of phosphoric acid present as dihydrogen salt, as above stated, cannot be used as a measure of the acidity, none of the older methods suggested for the estimation of this portion of the phosphoric acid is suited for acidity determinations.

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[1] See Winterberg, Zeitschr. f. physiol. Chem., **25**, and J. Baer, Arch. f. exp. Path. u. Pharm., **54**.

[2] Eppinger, Zeitschr. f. exp. Path. u. Therap., **8**; with Tedesko, Bioch. Zeitschr., **16**; Pohl. *ibid.*, 18; Staal, Zeitschr. f. physiol. Chem., **58**; Bostock, *ibid.*, **84**; A. Loewy, Centralbl. f. Physiol., **20**; 337.

We now determine the acidity simply by acidimetric methods, titrating with N/10 caustic alkali, using phenolphthalein as an indicator (NAEGELI, HÖBER, FOLIN). On account of the color of the urine and the presence of ammonium salts and alkaline earths, this method cannot yield entirely exact results. The greatest error is due to the alkaline earths, which, on titration with caustic alkali, precipitate as earthy phosphates in variable amounts and of variable composition. This error can be prevented, according to FOLIN, by the addition of neutral potassium oxalate, which precipitates the lime, and in this way the disturbing action of the ammonium salts is also inhibited. Perfectly accurate results are not obtained by this method, but it is the best of those which have been suggested.

It is performed as follows: 25 cc. of urine are placed in an Erlenmeyer flask (about 200 cc. capacity), treated with 1–2 drops of ½-per cent phenolphthalein solution, and shaken with 15–20 grams of powdered potassium oxalate and immediately titrated with N/10 caustic soda with constant shaking until a pronounced pale-rose color appears. VOZÁRIK [1] titrates the diluted urine without the addition of oxalate and uses phenolphthalein as indicator.

The acidity, as determined by titration, varies considerably under physiological conditions, but calculated as hydrochloric acid it amounts in man to about 1.5–2.3 grams in the twenty-four hours.

By titration we learn the amount of hydrogen present which can be substituted by a metal, i.e., the acidity in the ordinary older sense, but not the true acidity, the ion acidity, which is given by the concentration of the hydrogen ions of the urine. For similar reasons, as previously indicated in treating of the alkalinity of the blood-serum (page 272), the ion acidity cannot be determined by titration, while it can be determined according to the principle of the electrometric gas-chain method as there given. Such estimations have been made by v. RHORER and by HÖBER. For normal urine v. RHORER found as a minimum 4×10^{-7}, as a maximum 76×10^{-7}, and as an average 30×10^{-7}. HÖBER found 4.7×10^{-7}, 100×10^{-7}, and 49×10^{-7}, respectively. On an average the urine therefore contains 30–50 grams of hydrogen ions in 10 million liters. HENDERSON [2] has obtained much lower values, namely 10.10^{-7} as the average of 50 investigations, and has rather great differences for different persons. From the comparative estimation of the titration

[1] In regard to the degree of acidity and its estimation see Naegeli, Zeitschr. f. physiol. Chem., **30**; Höber, Hofmeister's Beiträge, 3; Folin, Amer. Journ. of Physiol., **9**; Vozárik, l. c.; de Jager, Zeitschr. f. physiol. Chem., **55**; and Ringer, *ibid.*, **60**; Grimbert and Morel, Compt. Rend., **154**.

[2] v. Rhorer, Pflüger's Arch., **86**; Höber, l. c. See also Jolles, Bioch. Zeitschr., **18**; Henderson, Bioch. Zeitschr., **24**.

acidity and the ion acidity it follows that no direct relation exists between these and that the extent of these two acidities may be independent of each other.

The **osmotic pressure** of the urine varies considerably even under physiological conditions. The limit for the freezing-point depression has been found by a number of investigators to be Δ 1.3° to 2.3° C. After partaking of considerable water it may be markedly lower, and on diminished supply of water it may be considerably higher.

In regard to the further physical-chemical imvestigations of the urine and as to the conclusions drawn from a combination of the chemical and the physico-chemical investigations of the urine, we must refer to the extensive work of CARL NEUBERG.[1]

The **specific gravity** of urine, which is dependent upon the relation existing between the quantity of water secreted and the solid urinary constituents, especially the urea and sodium chloride, may vary considerably, but is generally 1.017–1.020. After drinking large quantities of water it may fall to 1.002, while after profuse perspiration or after drinking very little water it may rise to 1.035–1.040. In new-born infants the specific gravity is low, 1.007–1.005. The determination of the specific gravity is an important means of learning the average amount of solids eliminated from the organism in the urine, and on this account the determination becomes of true value only when at the same time the quantity of urine voided in a given time is determined. The different portions of urine voided in the course of the twenty-four hours are collected, mixed together, the total quantity measured, and then the specific gravity taken.

The *determination of the specific gravity* is most accurately obtained with the pycnometer. For ordinary cases the specific gravity may be determined with sufficient accuracy by means of areometers. The areometers found in the trade, or *urinometers*, are graduated from 1.000 to 1.040; for exact observations it is better to use two urinometers, one graduated from 1.000 to 1.020, and the other from 1.020 to 1.040.

To determine the specific gravity of urine, if necessary filter the urine, or if it contains a urate sediment, first dissolve it by gentle heat, then pour the clear urine into a dry cylinder, avoiding the formation of froth. Air bubbles or froth, when present, must be removed with a glass rod or filter-paper. The cylinder, which should be about four-fifths full, must be wide enough to allow the urinometer to swim freely in the liquid without touching the sides. The cylinder and urinometer should both be dry or previously washed with the urine. On reading, the eye is brought on a level with the lower meniscus—which occurs when the surface of the liquid and the lower limb of the meniscus coincide; the read-

[1] Der Harn sowie die übrigen Ausscheidungen und Körperflüssigkeiten von Mensch und Tier. Teil. 2, Berlin, 1911.

ing is then made from the point where this curved line coincides with the scale of the urinometer. If the eye is not in the same horizontal plane with the convex line of the meniscus, but is too high or too low, the surface of the liquid assumes the shape of an ellipse, and the reading in this position is incorrect. Before reading, press the urinometer gently down into the liquid and then allow it to rise, and wait until it is at rest.

Each urinometer is graduated for a certain temperature, which, at least in the case of the better ones, is marked on the instrument. If the urine is not at the proper temperature, the following corrections must be made: For every three degrees above the normal temperature one unit of the last order is added to the reading, and for every three degrees below the normal temperature one unit (as above) is subtracted from the specific gravity observed. For example, when a urinometer graduated for 15° C. shows a specific gravity of 1.017 at 24° C., then the specific gravity at 15° C. = 1.017 + 0.003 = 1.020.

When great exactitude is required, as, for instance, a determination to the fourth decimal point, we make use of a urinometer constructed by LOHNSTEIN.[1] JOLLES[2] has also devised a small urinometer for the determination of the specific gravity of small amounts of urine, 20–25 cc. The specific gravity may also be determined by the WESTPHAL hydrostatic balance.

II. ORGANIC PHYSIOLOGICAL CONSTITUENTS OF URINE.

Urea, $\overset{+}{\text{U}}\text{r}$, $CON_2H_4 = CO\langle^{NH_2}_{NH_2}$, has been synthetically prepared in several ways, especially, as WÖHLER showed in 1828, by the metameric transformation of ammonium isocyanate: $CO.N.NH_4 = CO(NH_2)_2$. It is also produced by the decomposition or oxidation of certain bodies found in the animal organism, such as purine bodies, creatine, arginine, other amino-acids, and other substances.

Urea is found most abundantly in the urine of carnivora and man, but in smaller quantities in that of herbivora. In carnivora (dog) the urea nitrogen by abundant protein feeding may amount to 97–98 per cent of the total nitrogen of the urine (SCHÖNDORFF[3]). The quantity in human urine is ordinarily 20–30 p. m. It has also been found in small quantities in the urine of amphibians, fishes, and certain birds. Urea occurs in the perspiration in small quantities, and as traces in the blood and in most of the animal fluids. It also occurs in rather large quantities in the blood, liver, muscle,[4] and bile[5] of sharks, even in rather large quantities. Urea is also found in certain tissues and organs of mammals, especially

[1] Pflüger's Arch., **59**; Chem. Centralbl., 1895, **1**, and 1896, **2**.
[2] Wien. med. Presse, 1897, No. 8
[3] Pflüger's Arch., **117**.
[4] v. Schroeder, Zeitschr. f. physiol. Chem., **14**.
[5] Hammarsten, *ibid.*, **24**.

in the liver, spleen, muscles and others, although only in small amounts. Under pathological conditions, as in obstructed excretion, urea may appear to a considerable extent in the animal fluids and tissues.

The quantity of urea which is voided in twenty-four hours on a mixed diet is in a grown man about 30 grams, in women somewhat less. While children void less, the excretion relative to their body weight is greater than in grown persons. The physiological significance of urea lies in the fact that this body forms in man and carnivora, from a quantitative standpoint, the most important nitrogenous end-product of the metabolism of protein bodies. On this account the elimination of urea varies to a great extent with the catabolism of the protein, and above all with the quantity of absorbable proteins in the food ingested. The elimination of urea is greatest after an exclusive meat diet, and lowest, indeed less than during starvation, after the consumption of non-nitrogenous substances, since these diminish the metabolism of the proteins of the body.

If the consumption of the proteins of the body is increased, then the elimination of nitrogen is correspondingly increased. This is found to be the case in fevers, after poisoning with arsenic, antimony, phosphorus, and other protoplasmic poisons, and when there is a diminished supply of oxygen—as in severe and continuous dyspnœa, poisoning with carbon monoxide, hemorrhage, etc. In these cases it used to be considered that the rise in the excretion of nitrogen was due to an increased elimination of urea, because no exact difference was made between the quantity of urea and of total nitrogen in the urine. Recent researches have conclusively demonstrated the untrustworthiness of these observations. Since PFLÜGER and BOHLAND have shown that 16 per cent of the total nitrogen of the urine exists under physiological conditions in other compounds, not urea, attention has been called to the relation of the different nitrogenous constituents of the urine to each other, and it has been found, under pathological conditions, that this relation may vary considerably, especially in regard to the urea. We have numerous determinations by different investigators,[1] on the relation of the different nitrogenous constituents to each other in the normal urine of adults.

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[1] Pflüger and Bohland, Pflüger's Arch., **38** and **43**; Bohland, *ibid.*, **43**; Schultze, *ibid.*, **45**; Camerer, Zeitschr. f. Biologie, **24**, **27**, and **28**; Voges, Ueber die Mischung der stickstoffhaltigen Bestandtheile im Harn. etc. (Inaug.-Diss. Berlin. 1892), cited from Maly's Jahresber., **22**; K. Mörner and Sjöqvist, Skand. Arch. f. Physiol., **2**. See also Sjöqvist, Nord. Med. Arkiv., 1892, No. 36, and 1894, No. 10; Gumlich, Zeitschr. f. physiol. Chem., **17**; Bödtker, see Maly's Jahresber., **26**; Folin, Amer. Journ. of Physiol., **13**; Osterberg and Wolff, Journ. of biol. Chem., **3**; Haskins, *ibid.*, **2**; Donsé et Lambling, Journ. de Physiol. et de Path., **5**; Bouchet, *ibid.*, **14**; Lambling et Bouchet, Compt. rend. soc. biol., **71**; Long and Gephart, Journ. Amer. Chem. Soc., **34**.

Thus LONG and GEPHART found in the urine of six healthy men to whom the same qualitative diet was fed for a long time, the following division of the nitrogen in percentage of the total nitrogen: urea 79.87–84.34, creatinine 5.21–6.87, ammonia 3.6–4.74, uric acid 1.57–1.99, purine 0.33–0.96 and rest nitrogen 4.23–6.01 per cent. SJÖQVIST has made similar determinations on new-born babes from 1 to 7 days old. From all these analyses we obtain the following figures (*A* for adults and *B* for new-born babes). Of the total nitrogen there exists:

	A. Per Cent.	*B.* Per Cent.
Urea	84–91	73–76
Ammonia	2–5	7.8–9.6
Uric acid	1–3	3.0–8.5
Remaining nitrogenous substances	7–12	7.3–14.7

The variable relation between uric acid, ammonia, and urea nitrogen in children and adults is remarkable, since the urine of children is considerably richer in uric acid and ammonia, and considerably poorer in urea, than the urine of adults. A much larger number of analyses of children's urine is necessary to explain the division of the nitrogen therein. The absolute quantity of urea nitrogen in adults amounts to about 10–16 grams per day. In disease the proportion of the nitrogenous substances may be markedly changed, and a decrease in the quantity of urea and an increase in the quantity of ammonia have been observed in certain diseases of the liver. This will be considered in detail in connection with the formation of urea in the liver. It is natural that there should be a diminished formation of urea after a decrease in the ingestion of proteins or in a lowered catabolism. In diseases of the kidneys which disturb or destroy the integrity of the epithelium of the convoluted urinary tubules, the elimination of urea is considerably diminished.

Recently by means of PFAUNDLER'S [1] method, by precipitating the urine with phosphotungstic acid and closely studying the precipitate as well as the filtrate, it has been possible to learn further about the division of the nitrogen of the urine. We determine *a*, the total nitrogen; *b*, the nitrogen of the phosphotungstate precipitate; and *c*, the nitrogen in the filtrate from the phosphotungstate precipitate. This last contains the urea, hippuric acid, oxyproteic acids, and other bodies whose nitrogen is ordinarily designated as monamino-acid nitrogen. The urea nitrogen is especially determined. The bodies precipitated by phosphotungstic acid are not all known; but uric acid and purine bases, ammonia, creatinine, pigments, diamino-acids, diamines and ptomaines (if they occur), sulphocyanides, carbamic acid, urine mucoid, and proteid belong to this group. Special methods have been suggested for the determination of several of these substances (see below).

The urea nitrogen is always the greatest part of the total nitrogen, but otherwise the divisicn of the nitrogen undergoes considerable varia-

[1] Zeitschr. f. physiol. Chem., **30**.

tion and very great variations seem to occur not only in the healthy individual, but also and to a greater degree in diseased conditions.[1]

Formation of Urea in the Organism. The older statements of BÉCHAMP that urea is directly formed from proteins by oxidation has been denied by several investigators but according to recent statements of FOSSE[2] this is correct. On the hydrolysis of proteins arginine is found among other products, and as it is also produced in tryptic digestion, it is possible that a small portion of the urea is produced in this manner, varying according to the kind of protein. DRECHSEL claims that about 10 per cent of the urea can be accounted for in this way.

The possibility of a formation of urea from arginine has gained in interest since KOSSEL and DAKIN have discovered the presence of an enzyme, *arginase*, in the liver and other organs, which has the power of splitting arginine with the formation of urea. THOMPSON[3] has given a direct proof for the formation of urea from arginine. The introduction of arginine into the body of a dog either per os or subcutaneously has in his experiments led to an elimination of urea. While outside of the body only one-half of the nitrogen of arginine is split off as urea and the other half as ornithine, in the above experiments the increase in urea in several instances corresponded to the greater part if not the whole of the nitrogen of the arginine introduced. This increased formation of urea makes it probable that also ornithine is deamidized and the urea is formed from the ammonia split off.

By the action of alkalies, as above mentioned (Chapter X), urea may be formed from creatinine; still such an origin of urea in the animal body has not thus far been proved.

The amino-acids are considered as special mother-substances of urea. By numerous, generally older experiments with these acids, it has been proved that the amino-acids of the animal body are transformed in part into urea. The investigations by SALASKIN with the three amino-acids, glycocoll, leucine, and aspartic acid, have unmistakably shown that the surviving dog-liver, supplied with arterial blood, has the property of transforming the above amino-acids into urea or a closely allied substance.[4] Like the amino-acids the polypeptides are also transformed into

[1] See Satta, Hofmeister's Beiträge, **6**, which also gives the literature, and Erben, Zeitschr. f. Heilkunde, **25**.

[2] Compt. Rend., **154**.

[3] Kossel and Dakin, Zeitschr. f. physiol. Chem., **41**; Thompson, Journ. of Physiol., **32** and **33**.

[4] Schultzen and Nencki, Zeitschr. f. Biologie, 8; v. Knieriem, *ibid.*, **10**; Salkowski, Zeitschr. f. physiol. Chem., **4**; Salaskin, *ibid.*, **25**; Stolte, Hofmeister's Beiträge, **5**; Levene and Meyer, Amer. Journ. of Physiol., **25**; see also Loewi, Zeitschr. f. physiol. Chem., **25**; Richet, Compt. Rend., **118**, and Compt. rend. Soc. biol., **49**; Ascoli, Pflüger's Arch., **72**.

urea in the animal body, as shown by the investigations of ABDERHALDEN and his collaborators.[1]

There is no doubt that the ammonia formation is of great importance in the production of urea in the animal body.

A great number of older investigations[2] on the behavior of ammonium salts in the animal body have shown that not only ammonium carbonate, but also those ammonium salts which are burned into carbonate in the organism, are transformed into urea by carnivora as well as herbivora. v. SCHROEDER,[3] by irrigating the surviving dog's liver with blood treated with ammonium carbonate or ammonium formate, has shown that the formation of urea takes place, at least in part, in this organ. NENCKI, PAWLOW, ZALESKI and SALASKIN[4] have also found that, in dogs, the quantity of ammonia in the blood from the portal vein is considerably greater than that from the hepatic vein, and they claim that the liver retains in great part the ammonia thus supplied. The formation of urea from ammonia in the liver is a positively proved fact.

The assumption of a splitting off of ammonia from amino-acids stands in agreement with the experience that a deamidation of the amino-acids takes place in the animal body. The ammonia split off finds, in the blood and tissues, the carbon dioxide necessary for the formation of carbonate, and the investigations of NOLF, as well as those of MACLEOD and HASKINS,[5] on the equilibrium of carbonate and carbamate solutions and the conditions for the formation of both salts, must also be abundant evidence of a carbamate formation.

Important observations have been made which give support to the views of SCHULTZEN and NENCKI,[6] namely, that the amino-acids are transformed into urea with ammonium carbamate, $H_4N.O.CO.NH_2$, as an intermediate step. DRECHSEL has shown that the amino-acids yield carbamic acid by oxidation in alkaline fluid outside of the organism, and he obtained urea from ammonium carbamate by alternate oxidation and reduction. Carbamate has also been found in the blood (DRECHSEL) as well as in the urine (DRECHSEL, ABEL and MUIRHEAD)[7] and NENCKI

[1] Abderhalden with Teruuchi and with Babkin, Zeitschr. f. physiol. Chem., **47**, with Schittenhelm, *ibid.*, **51**.

[2] v. Knieriem, Zeitschr. f. Biologie, **10**; Feder, *ibid.*, **13**; Salkowski, Zeitschr. f. Biologie, **1**; Munk, *ibid.*, **2**; Coranda, Arch. f. exp. Path. u. Pharm., **12**; Schmiedeberg and Walter, *ibid.*, **7**; Hallervorden, *ibid.*, **10**; Pohl and Münzer, Arch., f. exp. Path. u. Pharm., **43**.

[3] Arch. f. exp. Path. u. Pharm., **15**. See also Salomon, Virchow's Arch., **97**.

[4] Arch. des sciences biol. de St. Pétersbourg, **4**; see also Chapter V, p. 336.

[5] Nolf, Zeitschr. f. physiol. Chem., **23**; Macleod and Haskins, Journ. of biol. Chem., **1**.

[6] Zeitschr. f. Biologie, 8.

[7] Drechsel, Ber. d. sächs. Gesellsch. d. Wissensch., 1875. See also Journ. f. prakt. Chem. (N. F.), **12**, **16**, and **22**; Abel, Arch. f. (Anat. u.) Physiol., 1891; Abel and Muirhead, Arch. f. exp. Path. u. Pharm., **31**.

and HAHN have made further observations on dogs with Eck's fistula, which substantiate this view. In such fistula dogs, they observed that when meat was fed, violent poisonous symptoms developed which were almost identical with those produced when carbamate was introduced into the blood. The same symptoms also appeared on the introduction of carbamate into the stomach of the fistula animal, while the introduction of carbamate into the stomach of a normal dog had no action.[1] As these observers also found that the urine of the dog on which the operation was made was richer in carbamate than that of the normal dog, they concluded that the symptoms were due to the non-transformation of the ammonium carbamate into urea in the liver, and they consider the ammonium carbamate as the substance from which the urea is derived in the mammalian liver.

Besides the above view of the formation of urea from ammonium carbonate and carbamate, which has been called the anhydride theory, we also have the oxidation theory of HOFMEISTER.

F. HOFMEISTER[2] found in the oxidation of different members of the fatty series, as well as in amino-acids and proteins, that urea was formed in the presence of ammonia, and he therefore suggests the possibility that urea may be formed by an oxidation-synthesis. According to him, in the oxidation of nitrogenous substances a radical $CONH_2$, containing the amide group, unites at the moment of formation with the radical NH_2 remaining on the oxidation of ammonia, forming urea.

Besides the above-mentioned theories as to the formation of urea, there are others which will not be given, because the only theory which has thus far been positively demonstrated is the formation of urea in the liver from ammonium compounds and amino-acids.

The liver is the only organ in which, up to the present time, a formation of urea has been directly detected;[3] and the question arises, what importance has this urea formation which takes place in the liver? Is the urea wholly or chiefly formed in the liver?

If the liver is the only organ capable of forming urea, it is to be expected, on the extirpation or atrophy of that organ, that a reduced

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[1] Hahn, Massen, Nencki et Pawlow, La fistule d'Eck de la veine cave inférieure et de la veine porte, etc. Arch. des sciences biol. de St. Pétersbourg, **1**, No. 4, 1892. In regard to certain differences between the symptoms with carbamate poisoning and after meat feeding with Eck fistula dogs, see Rothberger and Winterberg, Zeitschr. f. exp. Path. u. Therap., **1**; Hawk, Amer. Journ. of Physiol., **21**.

[2] Arch. f. exp. Path. u. Pharm., **37**.

[3] In regard to the investigations of Prevost and Dumas, Meissner, Voit, Gréhant, Gscheidlen and Salkowski, and others, on the rôle of the kidneys in the formation of urea, see v. Schroeder, Arch. f. exp. Path. u. Pharm., **15** and **19**, and Voit, Zeitschr. f. Biologie, **4**.

or, in short experiments, at least a strongly diminished elimination of urea should occur. As at least a part of the urea is formed in the liver from ammonium compounds, a simultaneous increase in the elimination of ammonia is to be expected.

The extirpation and atrophy experiments made on animals by different methods [1] have shown that sometimes a rather marked increase of ammonia and a diminished elimination of urea takes place after the operation, but that there are also cases in which, irrespective of the pronounced atrophy, an abundant formation of urea occurs, and no appreciable, if any, change in the proportion of ammonia to the total nitrogen and urea is observed. After shutting out from the circulation the organs of the posterior part of the body, especially the liver and kidneys, KAUFMANN [2] also found an important increase in the urea of the blood, and these different observations show that the liver is not the only organ, in the various animals experimented upon, in which urea is formed.

The observations made by numerous investigators [3] on human beings with cirrhosis of the liver, acute yellow atrophy of the liver, and phosphorus poisoning have led to the same result. These investigations teach that in certain cases the proportion of the nitrogenous substances may be so changed that urea is only 50–60 per cent of the total nitrogen, while in other cases, on the contrary, even in very extensive atrophy of the liver-cells, the formation of urea is not diminished, neither is the proportion between the total nitrogen, urea, and ammonia essentially changed. Even in the cases in which the formation of urea was relatively diminished and the elimination of ammonia considerably increased, further investigation must be instituted before it will be possible to assume a reduced ability of the organism to produce urea. An increased elimination of ammonia may, as shown by MÜNZER in the case of acute phosphorus poisoning, be dependent upon the formation of abnormally large quantities of acids, caused by abnormal metabolism, and these acids require a greater quantity of ammonia for their neutralization according to the law of elimination of ammonia. That an abnormal formation

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[1] Nencki and Hahn, l. c.; Slosse, Arch. f. (Anat. u.) Physiol., 1890; Lieblein, Arch. f. exp. Path. u. Pharm., **33**; Nencki and Pawlow, Arch. des science. biol. de St. Pétersbourg, **5**. See also v. Meister, Maly's Jahresber., **25**; Salaskin and Zaleski, Zeitschr. f. physiol. Chem., **29**; Fischler and Bardach, *ibid.*, **78**.

[2] Compt. rend. soc. biol., **46**, and Arch. de Physiol. (5), **6**.

[3] See Hallervorden, Arch. f. exp. Path. u. Pharm., **12**; Weintraud, *ibid.*, **31**; Münzer and Winterberg, *ibid.*, **33**; Stadelmann, Deutsch. Arch. f. klin. Med., **33**; Fawitzki, *ibid.*, **45**; Münzer, *ibid.*, **52**; Fränkel, Berlin. klin. Wochenschr., 1878; Richter, *ibid.*, 1896; Mörner and Sjöqvist, Skand. Arch. f. Physiol., **2**, and Sjöqvist, Nord. Med. Arkiv, 1892; Gumlich, Zeitschr. f. physiol. Chem., **17**; v. Noorden, Lehrb. d. Pathol. des Stoffwechsels, 2. Aufl., Bd. 1, 104.

of acid occurs after the cutting out of the liver has been especially shown by SALASKIN and ZALESKI.[1]

For the present we are not justified in the statement that the liver is the only organ in which urea is formed, and only continued investigation can yield further information as to the extent and importance of the formation of urea, from ammonium compounds, in the liver.

Properties and Reactions of Urea. Urea crystallizes in needles or in long, colorless, four-sided, often hollow, anhydrous rhombic prisms. It has a neutral reaction, and produces a cooling sensation on the tongue like saltpeter. It melts at 132° C. At ordinary temperatures it dissolves in an equal weight of water and in five parts alcohol; it requires one part boiling alcohol for solution; it is insoluble in alcohol-free anhydrous ether, and also in chloroform. If urea in substance is heated in a test-tube, it melts, decomposes, gives off ammonia, and finally leaves a non-transparent white residue which, among other substances, contains cyanuric acid and *biuret*, which latter dissolves in water, giving a beautiful reddish-violet liquid with copper sulphate and alkali (*biuret reaction*). On heating with baryta-water or caustic alkali, also in the so-called alkaline fermentation of urine caused by micro-organisms, urea splits into carbon dioxide and ammonia with the addition of water. The same decomposition products are produced when urea is heated with concentrated sulphuric acid. An alkaline solution of sodium hypobromite decomposes urea into nitrogen, carbon dioxide, and water according to the equation

$$CON_2H_4+3NaOBr=3NaBr+CO_2+2H_2O+N_2.$$

With a concentrated solution of furfurol and hydrochloric acid, urea in substance gives a coloration passing from yellow, green, blue, to violet, and then after a few minutes beautiful purple-violet (SCHIFF'S reaction). According to HUPPERT[2] the test is best performed by taking 2 cc. of a concentrated furfurol solution, 4–6 drops of concentrated hydrochloric acid, and adding to this mixture, which must not be red, a small crystal of urea. A deep violet coloration appears in a few minutes.

Urea forms crystalline compounds with many acids. Among these the one with nitric acid and the one with oxalic acid are the most important.

UREA NITRATE, $CO(NH_2)_2.HNO_3$. On crystallizing quickly this compound forms thin rhombic or six-sided overlapping tiles, or colorless

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[1] Zeitschr. f. physiol. Chem., 29.

[2] Huppert-Neubauer, Analyse des Harns, 10. Aufl., 296.

plates, with an angle of 82°. When crystallizing slowly, larger and thicker rhombic pillars or plates are obtained. This compound is rather easily soluble in pure water, but is considerably less soluble in water containing nitric acid; it may be obtained by treating a concentrated solution of urea with an excess of strong nitric acid free from nitrous acid. On heating this compound it volatilizes without leaving a residue.

This compound may be employed with advantage in detecting small amounts of urea. A drop of the concentrated solution is placed on a microscope slide and the cover-glass placed upon it; a drop of nitric acid is then placed on the side of the cover-glass and allowed to flow under. The formation of crystals begins where the solution and the nitric acid meet. Alkali nitrates may crystallize very similarly to urea nitrate when they are contaminated with other bodies; therefore, in testing for urea, the crystals must be identified as urea nitrate by heating and by other means.

UREA OXALATE, $2.CO(NH_2)_2.H_2C_2O_4$. This compound is more sparingly soluble in water than the nitric-acid compound. It is obtained in rhombic or six-sided prisms or plates on adding a saturated oxalic-acid solution to a concentrated solution of urea.

Urea also forms combinations with mercuric nitrate in variable proportions. If a very faintly acid mercuric-nitrate solution is added to a 2 per cent solution of urea and the mixture carefully neutralized, a compound is obtained of a constant composition which contains for every 10 parts of urea 72 parts of mercuric oxide. This compound serves as the basis of LIEBIG'S titration method. Urea also combines with salts, forming mostly crystallizable combinations, as, for instance, with sodium chloride, with the chlorides of the heavy metals, etc. An alkaline but not a neutral solution of urea is precipitated by mercuric chloride.

If urea is dissolved in dilute hydrochloric acid and then an excess of formaldehyde is added, a thick, white, granular precipitate is obtained which is difficultly soluble and whose composition is somewhat disputed.[1] With phenylhydrazine, urea in strong acetic acid gives a colorless crystalline compound of phenylsemicarbazid, $C_6H_5NH.NH:CONH_2$, which is soluble with difficulty in cold water and melts at 172° C. (JAFFÉ [2]).

The method of preparing urea from urine is in the main as follows: Concentrate the urine, which has been faintly acidified with sulphuric acid, at a low temperature, add an excess of nitric acid, at the same time keeping the mixture cool, press the precipitate well, decompose it in water with freshly precipitated barium carbonate, dry on the water-bath, extract the residue with strong alcohol, decolorize when necessary with animal charcoal, and filter while warm. The urea which crystallizes

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[1] See Tollens and his pupils, Ber. d. deutsch. chem. Gesellsch., **29**, 2751; Goldschmidt, *ibid.*, **29**; and Chem. Centralbl., 1897, **1**, 33; Thoms, *ibid.*, **2**, 144 and 737.

[2] Zeitschr. f. physiol. Chem., **22**.

on cooling is purified by recrystallization from warm alcohol. A further quantity of urea may be obtained from the mother-liquor by concentration. The urea is purified from contaminating mineral bodies by redissolving in alcohol-ether. If it is only necessary to detect the presence of urea in urine, it is sufficient to concentrate a little of the urine on a watch-glass and, after cooling, treat it with an excess of nitric acid. In this way we obtain crystals of urea nitrate.

Quantitative Estimation of the Total Nitrogen and Urea in Urine. Among the various methods proposed for the estimation of the total nitrogen, that suggested by KJELDAHL is to be recommended. LIEBIG'S method for the estimation of urea is really a method for determining the total nitrogen, but as it is very seldom used now, we can refer to larger works in regard to details.

KJELDAHL'S method consists in transforming all the nitrogen of the organic substances into ammonia by heating with a sufficiently concentrated sulphuric acid. The ammonia is distilled off, after supersaturating with alkali, and collected in standard sulphuric acid. The following reagents are necessary:

1. *Sulphuric Acid.* Either a mixture of equal volumes of pure concentrated and fuming sulphuric acid, or else a solution of 200 grams phosphoric anhydride in 1 liter of pure concentrated sulphuric acid. 2. *Caustic soda* free from nitrates, 30–40 per cent solution. The quantity of this caustic-soda solution necessary to neutralize 10 cc. of the acid mixture must be determined. 3. Metallic *mercury* or pure yellow *mercuric oxide.* (The addition of this facilitates the destruction of the organic substances.) 4. A *potassium-sulphide* solution of 4 per cent, whose object is to decompose any mercuric amide combination which might not evolve its ammonia completely during the distillation with caustic soda. 5. 1/5 normal sulphuric acid and 1/5 normal caustic soda solution.

In performing the determination 5 cc. of the carefully measured and filtered urine are placed in a long-necked KJELDAHL flask, a drop of mercury or about 0.3 gram of mercuric oxide added, and then treated with 10–15 cc. of the strong sulphuric acid. The contents are heated very carefully, placing the flask at an angle, until they just begin to boil gently; this is continued for about half an hour after the mixture becomes colorless. On cooling, the contents are transferred to a voluminous distilling-flask, carefully washing the KJELDAHL flask with water and the greater part of the acid is neutralized by caustic soda. A few zinc shavings are added to prevent too rapid ebullition on distillation, and then an excess of caustic-soda solution which has previously been treated with 30–40 cc. of the potassium-sulphide solution. The flask is quickly connected with the condenser-tube and all the ammonia distilled off. In order to prevent loss of ammonia it is best to lower the end of the exit-tube below the surface of the acid, the regurgitation of the acid being prevented by having a bulb blown on the exit-tube. Not less than 25–30 cc. of the standard acid is used for every 5 cc. of urine, and on completion of the distillation the acid is retitrated with 1/5 normal caustic soda using rosolic acid, tincture of cochineal, or lacmoid as indicator. Each cubic centimeter of the acid corresponds to 2.8 milligrams nitrogen. As a control and in order to test the purity of the reagents, or to eliminate any error caused by an accidental quantity of ammonia in the air, we always make a blank determination with the reagents.

Recently FOLIN and FARMER [1] have suggested a method for the estimation of the total nitrogen in very small quantities of urine, 1 cc. dilute urine. After hydrolysis with acid the ammonia formed is colorimetrically determined by means of NESSLER'S reagent.

Among the methods suggested for the special estimation of urea, that of MÖRNER-SJÖQVIST, in combination with FOLIN'S method, is the one that is generally used.

Principle of Mörner-Sjöqvist's Method.[2] According to this method the nitrogenous constituents of the urine, with the exception of urea, ammonia, hippuric acid, creatinine, and traces of allantoin,[3] are precipitated by a mixture of alcohol and ether after the addition of a solution of barium chloride and barium hydroxide, or in the presence of sugar with solid barium hydroxide. The urea is determined in the concentrated filtrate, after driving off the ammonia, by KJELDAHL'S nitrogen estimation. The slight error due to the presence of hippuric acid and creatinine can be prevented according to MÖRNER by a combination of his method with FOLIN'S method.

Principle of Folin's Method.[4] On heating urea with hydrochloric acid and crystalline magnesium chloride, which melts in its water of crystallization at 112–115° C. and then boils at about 150–155° C., the urea is completely decomposed, while no appreciable decomposition of the hippuric acid and creatinine takes place. The ammonia produced from the urea is distilled off and determined by titration. The amount of ammonia previously existing in the urine must be specially determined.

Determination of Urea by the Mörner-Sjöqvist and Folin Method.[5] Five cc. of the urine are treated with 1.5 grams of powdered barium hydroxide, and when as much of this is dissolved as possible by gently mixing, it is precipitated by 100 cc. of the alcohol and ether mixture (⅓ vol. ether). On the following day it is filtered and the precipitate washed with the alcohol and ether mixture. The alcohol and ether are distilled off from the filtrate at about 55° C. (not above 60° C.). The remaining liquid is treated with 2 cc. of hydrochloric acid of sp.gr. 1.124 (for 5 cc. urine), and carefully transferred to a flask of 200 cc. capacity, and evaporated to dryness on the water-bath. Then add 20 grams of crytalline magnesium chloride to the contents of the flask and 2 cc. of concentrated hydrochloric acid, and boil on a wire gauze over a small flame for two hours, making use of a proper return cooler.

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[1] Journ. of biol. Chem., **11**.

[2] Skand. Arch. f. Physiol., **2**, and Mörner, *ibid.*, **14**, where the recent literature may also be found.

[3] According to Wiechowski, Hofmeister's Beiträge, **11**, the quantity of allantoin is so great in urine that it must be considered in this method.

[4] Zeitschr. f. physiol. Chem., **32**, **36** and **37**.

[5] See Mörner, Skand. Arch. f. Physiol., **14**.

After cooling it is diluted to about ¾ to 1 liter with water, the ammonia completely distilled off, after making it alkaline with caustic soda, and the ammonia collected in standard acid. After boiling in order to drive off the CO_2 and cooling, the acid is retitrated.

In recent years objections of various kinds have been made against these methods, which are directed towards their exactness and which have led to changes in several directions (BENEDICT and GEPHART, LEVENE and MEYER, GILL, ALLISON and GRINDLEY). These changes are: precipitation of the other nitrogenous substances (nearly all the ammonia) with phosphotungstic acid, decomposition of the urea in the filtrate by heating with acid in an autoclave to 150,° and distilling off the ammonia from the solution, made alkaline, not by boiling with alkali, but by the aid of a vacuum or by means of a current of air. These changes have been carefully studied by HENRIQUES and GAMMELTOFT[1] and they have suggested the following method:

Henriques and Gammeltoft Method. First determine in 5 cc. urine how much of a 10 per cent phosphotungstic acid solution (in N/2 H_2SO_4) is necessary to exactly cause a complete precipitation. Then place 10 cc. of the urine in a 100 cc. flask, add the determined quantity of phosphotungstic acid solution and fill the flask up to the 100 cc. mark with N/2 H_2SO_4. The liquid is allowed to stand after mixing until it has settled and it is then filtered. Two portions of 10 cc. each are placed in test-tubes of Jena glass, covered with tin-foil and placed in the autoclave at 150° C. for 1½ hours. The contents of the test-tubes are now placed in a flask, and the ammonia determined either by passing a current of air through it (after the addition of sodium carbonate) or by distillation in a vacuum (after the addition of barium hydrate dissolved in methyl alcohol). FOLIN and PETTIBONE[2] have suggested a method, according to which the ammonia is determined colorimetrically with Nessler reagent.

KNOP-HÜPNER'S method[3] is based on the fact that urea, by the action of sodium hypobromite, splits into water, carbon dioxide (which dissolves in the alkali), and nitrogen, whose volume is measured (see page 686). This method is less accurate than the preceding ones, and therefore in scientific work it is discarded. It is of value to the physician and for practical purposes, because of the ease and rapidity with which it may be performed, even though it may not give very accurate results. For practical purposes a number of different apparatus have been constructed to facilitate the use of this method.

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[1] Benedict and Gephart, Journ. of Amer. Chem. Soc., **30**; Levene and Meyer, *ibid.*, **31**; Gill, Allison and Grindley, *ibid.*, **31**; Henriques and Gammeltoft, Skand. Arch. f. Physiol., **25**.

[2] Folin and Pettibone, Journ. of Biol. Chem., **11**.

[3] Knop, Zeitschr. f. analyt. Chem., **9**; Hüfner, Journ. f. prakt. Chem. (N. F.), **8**. In regard to the extensive literature, see Huppert-Neubauer, 10. Aufl., 304, and following. See also Keogh, Zeitschr. f. physiol. Chem., **84**.

In regard to other methods such as BUNSEN'S method with its many modifications as suggested by PFLÜGER, BOHLAND and BLEIBTREU, we refer to more complete handbooks.

For the quantitative estimation of urea in blood or other animal fluids, as well as in the tissues, SCHÖNDORFF has proposed a method where the proteins and extractives are first precipitated by a mixture of phosphotungstic acid and hydrochloric acid, and then the filtrate made alkaline with lime. The quantity of ammonia formed on heating a part of this filtrate to 150° C. with phosphoric acid and the amount of carbon dioxide produced by heating the other part to 150° C. are determined. In regard to the principles of this method, as well as to the details, we refer to the original article (PFLÜGER'S Arch., 62). SALKOWSKI[1] has recently suggested a method for estimating the urea in tissues.

Urein is the name given by OVID MOOR to a product which he obtained by extracting urine, which had been evaporated to a syrup, with absolute alcohol and precipitating the urea with alcohol containing oxalic acid, or by cooling and treatment with alcohol. Urein is a golden-yellow oil which is poisonous; it reduces permanganate in the cold, and it forms the chief portion of the nitrogenous extractives of urine. There is no doubt that urein is a mixture of several substances. According to MOOR,[2] the amount of urea in the urine is only about one-half that ordinarily given, and he has suggested a new method for the determination of the true quantity of urea. The possibility that in the urine we have other bodies besides urea which have been determined with the urea cannot be denied *a priori*. From the investigations published so far it must be said that MOOR'S assertions are not sufficiently grounded.[3]

Carbamic Acid, $CH_3NO_2 = CO\begin{<}NH_2\\OH\end{>}$. This acid is not known in the free state, but only as salts. Ammonium carbamate is produced by the action of dry ammonia on dry carbon dioxide, but also after the addition of Na_2CO_3 to a solution which contains an ammonium salt (MACLEOD and HASKINS). Carbamic acid is also produced by the action of potassium permanganate on protein and several other nitrogenous organic bodies.

The occurrence of carbamic acid in human and animal urines has already been considered in connection with the formation of urea. The calcium salt which is soluble in water and ammonia but insoluble in alcohol, is the most important in the detection of this acid. The solution of the calcium salt in water becomes cloudy on standing, but much more quickly on boiling, and calcium carbonate separates. NOLF, MACLEOD and HASKINS have made experiments as to the method of formation of carbamic acid. The latter have indicated a new method for the quantitative estimation of carbamates.[4]

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[1] Arbeiten aus dem pathol. Institute, Berlin, 1906.

[2] O. Moor, Bull. Acad. de St. Pétersbourg, **14** (also Maly's Jahresber., **31**, 415), and Zeitschr. f. Biologie, **44** and **45**, and Zeitschr. f. physiol. Chem., **40** and **48**.

[3] See Kuliabko, Maly's Jahresber., **31**, 415; Erben, Zeitschr. f. physiol. Chem., **38**; Folin, *ibid.*, **37**; Gies, Journ. Amer. Chem. Soc., **25**; Haskins, Amer. Journ. of Physiol., **12**; Lippich, Zeitschr. f. physiol. Chem., **48** and **52**.

[4] Nolf, Zeitschr. f. physiol. Chem., **23**; Macleod and Haskins, Amer. Journ. of Physiol., **12**, and Journ. of biol. Chem., **1**.

Carbamic-acid ethylester (urethane), as shown by JAFFÉ,[1] may pass, by the mutual action of alcohol and urea, into the alcoholic extract of urine when one is working with large quantities.

FOLIN [2] claims that all human urine contains a body which is probably *methyl-urea.*

Creatinine, $C_4H_7N_3O$, or $NH:C\begin{cases} NH\text{——}CO \\ \qquad\quad | \\ N(CH_3).CH_2 \end{cases}$, is the anhydride of

Creatine, $NH:C\begin{cases} NH_2 \\ N(CH_3).CH_2.COOH \end{cases}$, which occurs in the muscles, bird urine and sometimes also in human urine.

Creatinine occurs in human urine and in that of certain mammalia. It has also been found in ox-blood, milk, though in very small amounts, in meat extracts, and in the flesh of certain fishes.

The quantity of creatinine in human urine is, in a grown man voiding a normal quantity of urine in the course of a day, 0.6–1.3 grams (NEUBAUER), or on an average 1 gram. JOHNSON [3] found 1.7–2.1 grams per day, and similar results have been obtained by v. HOOGENHUYZE and VERPLOEGH.[4] The quantity of creatinine with a diet free from meat is, FOLIN [5] says, variable for different individuals, but is constant for the same person. He never found the quantity below 1 gram and often between 1.3 and 1.7 grams. Nurslings also eliminate creatinine, although the quantity is small (v. HOOGENHUYZE and VERPLOEGH). The quantity of creatinine nitrogen in per cent of the total nitrogen varies under different conditions, but is on an average about 4.5–6.9 per cent, as determined by several experimenters.

Creatine occurs especially in the urine of birds and also in the urine of nurslings, but also in older children (ROSE, FOLIN and DENIS). It has also been found in the urine of pregnant women (KRAUSE and CRAMER) but otherwise only in starvation, in diabetes, diseases of the liver, fevers and diseases accompanied by a destruction of the body proteins, especially muscle-proteins. Between creatine and creatinine elimination a relation exists it seems, at least for certain cases, namely with a decrease in the quantity of creatinine eliminated the quantity of creatine increases (LEVENE and KRISTELLER).[6]

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[1] Zeitschr. f. physiol. Chem., 14.

[2] Journ. of biol. Chem., 3.

[3] Huppert-Neubauer, Harnanalyse, 10. Aufl., 387.

[4] Zeitschr. f. physiol. Chem., 46.

[5] Amer. Journ. of Physiol. 13; af. Klercker, Hofmeister's Beiträge, 8.

[6] Rose, Journ. of biol. Chem., 10; Folin and Denis, *ibid.*, 11; Krause and Cramer, Journ. of Physiol., 40 (Proc. physiol. Soc., July, 1910, LXI); Schaffer, Amer. Journ. of Physiol., 23; Levene and Kristeller, *ibid.*, 24.

As the two bodies, creatine and creatinine, can easily be transformed into each other, it has been considered for a long time that the urinary creatinine is formed from the creatine of the muscles and other organs. Unfortunately the authorities disagree on this question. FOLIN in his investigations found that about 80 per cent of the creatinine introduced was again eliminated, while the creatine taken did not appear in the urine as creatinine, but was partly retained by the body and in part eliminated as such. An intravital transformation of creatine into creatinine is disputed by v. KLERCKER, MELLANBY and LEFMANN,[1] while it is accepted by GOTTLIEB, STANGASSINGER, S. WEBER, v. HOOGENHUYZE and VERPLOEGH and ROTHMANN. The observations of MYERS and FINE indicate a production of urinary creatinine from creatine, that is they found that the creatinine elimination by the urine in rabbits was greater according to the total creatine content of the respective animal. The investigations of PEKELHARING and v. HOOGENHUYZE on the behavior of parenterally introduced creatine in rabbits and dogs, show without any doubt that a part of the creatine is actually transformed into creatinine. TOWLES and VOEGTLIN[2] have also observed that the subcutaneously injected creatine increases somewhat the creatinine elimination, while this is not the case with creatine taken per os. The condition of the digestive apparatus also seems to be of importance here. PEKELHARING and v. HOOGENHUYZE found that in dogs of the parenterally introduced creatine always a smaller part (as creatine and creatinine) passed into the urine during the digestion than during rest of the digestive organs. They explain this by the accepted ability of the liver to partly destroy the creatine and partly by an anhydride formation of transforming the creatine into creatinine.

As mentioned in Chapter X the proteins and the guanidine groups therein are considered as the mother-substance of these two bodies. If the creatinine (creatine) originates from the protein it is evident that we must differentiate between food-protein and body-protein. The quantity of creatinine is, inasmuch as it is increased by meat diet, dependent upon the food; but otherwise, as found by FOLIN and in chief substantiated by others, is rather independent of the food. Its elimination does not run parallel with the urea and the total nitrogen, and consequently is not in general greater with food rich in protein than with food poor therein. On the contrary, its extent, as shown by other conditions, is dependent upon the intensity of the metabolism in the cells, especially the muscle

[1] Folin, Hammarsten's Festschrift, 1906; v. Klercker, Bioch. Zeitschr., **3**; Mellanby, Journ. of Physiol., **36**; Lefmann, Zeitschr. f. physiol. Chem., **57**.

[2] See footnote 1, page 574, and v. Hoogenhuyze and Verploegh, Zeitschr. f. physiol. Chem., **59**; Pekelharing and v. Hoogenhuyze, *ibid.*, 69; Towles and Voegtlin, Journ. of biol. Chem., **10**; Myers and Fine, *ibid.*, **14**.

tissue, and the creatinine, according to FOLIN, is a product of the endogenous protein metabolism.

Reports as to the behavior of the creatinine elimination with work are conflicting. v. HOOGENHUYZE and VERPLOEGH, who made use of a much more trustworthy method of quantitative estimation than their predecessors, find that muscular activity as a rule does not cause any rise in the creatinine elimination, and that in man such a rise with work occurs only when the body is obliged to live upon its own tissues. S. WEBER[1] also finds an absolute increase in the elimination of creatinine only in starving dogs. Other investigators could not find any increase in the elimination of creatinine by work, although such a rise was found as shown by PEKELHARING and HARKINK,[2] by the muscle tonus.

In starvation a decrease in the creatinine but a simultaneous increase in the elimination of creatine has been found in man (v. HOOGENHUYZE and VERPLOEGH, CATHCART, BENEDICT and MYERS[3]). Such an increase in the creatinine elimination only occurs in those conditions which are accompanied by acidosis, and correspondingly it can be prevented by the introduction of carbohydrates (CATHCART, MENDEL and ROSE) The creatinine elimination in certain cases has therefore been explained by a disturbed carbohydrate metabolism. This is neverthless on the other hand disputed by WOLF and OESTERBERG[4] who find that the creatinine elimination in starvation can be arrested by the introduction of proteins alone.

Little is known about the behavior of creatinine in disease, nor are the observations in accord. In anæmia and cachexia the elimination of creatinine is diminished, and when the metabolism is increased the elimination is also increased. That this is the case, at least in fevers, seems to be borne out by several concurrent observations.[5] In diseases of the liver a diminished elimination of creatinine may occur, and in cases of carcinoma of the liver considerable creatine has been found in the urine (v. HOOGENHUYZE and VERPLOEGH, MELLANBY). The role of the liver in the creatine-creatinine metabolism, is, as has already been mentioned in Chapter X, not clear. The exclusion of the liver from the metabolism of a dog with Eck fistula had no result in the experiments of TOWLES

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[1] Arch. f. exp. Path. u. Pharm., **58**. Further literature may be found in v. Hoogenhuyze and Verploegh, Zeitschr. f. physiol. Chem., **46**.

[2] Maillard and Clausmann, Journ. de Physiol. et de Path., **12**; Prayon, Maly's Jahresb., **40**; Pekelharing and Harkink, Zeitschr. f. physiol. Chem., **75**.

[3] v. Hoogenhuyze and Verploegh, Zeitschr. f. physiol. Chem., **57**; Cathcart, Bioch. Zeitschr., **6**; Benedict and Myers, Amer. Journ. of Physiol., **18**; Jaffé, l. c.

[4] Cathcart, Jour. of Physiol., **39**; Mendel and Rose, Journ. of biol. Chem., **10**; Wolf and Osterberg, Bioch. Zeitschr., **35**; Wolf, Journ. of biol. Chem., **10**.

[5] See O. af Klercker, Zeitschr. f. klin. Med., **68**.

and VOEGTLIN. The dogs, after feeding with creatine and creatinine, behaved like normal dogs, and the observations of other investigators such as LONDON and BOLJARSKI, FOSTER and FISHER[1] upon dogs with Eck fistula have not had any unanimous results or they are hard to explain.

Properties of Creatinine. Creatinine crystallizes in colorless, shining monoclinic prisms which differ from creatine crystals in not becoming white with loss of water when heated to 100° C. It dissolves in 11 parts cold water, but more easily in warm water. It is difficultly soluble in cold alcohol, but the reports in regard to its solubility differ widely.[2] It is more soluble in warm alcohol and nearly insoluble in ether. In alkaline solution creatinine is very easily converted into creatine on warming.

Creatinine gives an easily soluble crystalline compound with hydrochloric acid. A solution of creatinine acidified with mineral acids gives crystalline precipitates with phosphotungstic and phosphomolybdic acids even in very dilute solutions (1:10000), (KERNER, HOFMEISTER[3]). It is precipitated, like urea, by mercuric-nitrate solution and also by mercuric chloride. On treating a dilute creatinine solution with sodium acetate and then with mercuric chloride a precipitate of glassy globules having the composition $4(C_4H_7N_3O.HCl.HgO)3HgCl_2$ separates on standing some time (JOHNSON). Among the compounds of creatinine, that with zinc chloride, *creatinine-zinc chloride*, $(C_4H_7N_3O)_2ZnCl_2$, is of special interest. This combination is obtained when a sufficiently concentrated solution of creatinine in alcohol is treated with a concentrated, faintly acid solution of zinc chloride. Free mineral acids dissolve the compound, hence they must not be present; this, however, may be prevented by an addition of sodium acetate. In the impure state, as from urine, creatinine-zinc chloride forms a sandy, yellowish powder which under the microscope appears as fine needles, forming concentric groups, mostly complete rosettes or yellow balls or tufts, or grouped as brushes. On slowly crystallizing or when very pure, more sharply defined prismatic crystals are obtained. The compound is slightly soluble in water.

Creatinine acts as a reducing agent. Mercuric oxide is reduced to metallic mercury, and oxalic acid and methylguanidine (methyluramine) are formed. Creatinine also reduces cupric hydroxide in alkaline solution, forming a colorless soluble compound, and only after continued boiling with an excess of copper salt is free suboxide of copper formed. Creat-

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[1] Towles and Voegtlin, l. c.; London and Boljarski, Zeitschr. f. physiol. Chem., **62**; Foster and Fischer, Journ. of biol. Chem., **9**.

[2] See Huppert-Neubauer, 10. Aufl., and Hoppe-Seyler-Thierfelder's Handbuch. 8. Aufl.

[3] Kerner, Pflüger's Arch., **2**; Hofmeister, Zeitschr. f. physiol. Chem., **5**.

inine interferes with TROMMER'S test for sugar, partly because it has a reducing action, and partly by retaining the copper suboxide in solution. The compound with copper suboxide is not soluble in a saturated soda solution, and if a little creatinine is dissolved in a cold saturated soda solution and then a few drops of FEHLING'S reagent added, a white flocculent compound separates after heating to 50–60° C. and then cooling (v. MASCHKE'S[1] reaction). An alkaline bismuth solution (see Sugar Tests) is not reduced by creatinine.

An aqueous solution of creatinine is precipitated by picric acid. The precipitate consists on recrystallization from hot water, of thin, silky, pale yellow needles (JAFFÉ). If the urine is treated with picric acid (20 cc. of a 5 per cent solution in alcohol for each 100 cc. urine), then a double picrate of creatinine and potassium is precipitated (JAFFÉ). If a solution of creatinine in water (or urine) is treated with a watery solution of picric acid and a few drops of a dilute caustic-soda solution, a red coloration, lasting several hours, immediately occurs at the ordinary temperature, which turns yellow on the addition of acid (JAFFÉ'S[2] reaction). Acetone gives a more reddish-yellow color. Glucose gives with this reagent a red coloration only after heating. If we add a few drops of a freshly prepared very dilute sodium-nitroprusside solution (sp.gr. 1.003) to a dilute creatinine solution (or to the urine) and then a few drops of caustic soda, a ruby-red liquid is obtained which quickly turns yellow again (WEYL'S[3] reaction). If the cold yellow solution is neutralized and treated with an excess of acetic acid, a crystalline precipitate of a nitroso-compound ($C_4H_6N_4O_2$) of creatinine separates on stirring (KRAMM) or creatininoxim (SCHMIDT[4]). If, on the contrary, the yellow solution is treated with an excess of acetic acid and heated, the solution becomes first green and then blue (SALKOWSKI[5]); finally a precipitate of Prussian blue is obtained.

A reaction which in description is similar and which, although not solely (ARNOLD) but at least partially (HOLOBUT), appears after partaking of protein food or meat soup is ARNOLD'S reaction.[6] This reaction is due to an unknown endogenous metabolism product. If 10–20 cc. urine are treated with a few drops of a 4 per cent sodium nitroprusside solution and then with 5–10 cc. of a 5 per cent sodium or potassium hydroxide solution, at first a strong and pure violet color is obtained with an absorption band between D and E, then it becomes purple-red and then brown-red and finally yellow. On the addition of acetic

[1] Zeitschr. f. analyt. Chem., **17**.
[2] Zeitschr. f. physiol. Chem., **10**.
[3] Ber. d. deutsch. chem. Gesellsch., **11**.
[4] Kramm, Centralbl. f. d. med. Wissensch., 1897; Schmidt, cited from Chem. Centralbl., 1912, **2**.
[5] Zeitschr. f. physiol. Chem., **4**.
[6] Arnold, Zeitschr. f. physiol. Chem., **49** and **83**; Holobut, *ibid.*, **56**.

acid the violet or purple-red color passes into blue, which soon becomes pale and finally a pale yellow color. It differs from the creatinine in color and the absorption band as well as in that the creatinine reaction requires more sodium nitroprusside.

The best method for preparing creatinine is the following, suggested by FOLIN.[1] The creatinine is first precipitated as the double picrate of creatinine and potassium by means of picric acid according to JAFFÉ's method, and then this precipitate, while still moist, is decomposed by $KHCO_3$ and water. The solution, which contains the creatinine besides potassium carbonate and small amounts of impurities, is neutralized with sulphuric acid and the sulphate precipitated by alcohol. The creatinine is now converted into the double zinc-chloride salt and this last treated with moist lead hydroxide. After the removal of the lead, the solution contains a mixture of creatinine and creatine, which last is completely transformed into creatinine by heating for forty-eight hours with normal sulphuric acid. After exact neutralization with barium-hydroxide solution it is concentrated to the point of crystallization.

According to recent work of FOLIN and BLANCK the creatinine-zinc chloride can be dissolved in warm 10 per cent sulphuric acid when creatinine-zinc alum $(C_4H_7N_3O)_2SO_4ZnSO_4$, $8H_2O$ is obtained and from this the creatinine can be obtained by decomposing with barium acetate and removing the zinc by H_2S. Creatine can, according to FOLIN and DENIS,[2] be transformed into creatinine by heating in an autoclave for 3 hours under a pressure of 4–5 kg. per qcm.

The *quantitative estimation of creatinine* used to be performed according to NEUBAUER'S method for the preparation of creatinine, or more simply by SALKOWSKI'S [3] modification of this method. As this method is now seldom used we refer the reader to other hand-books.

FOLIN [4] has suggested a colorimetric method for determining creatinine which is based upon JAFFÉ'S picric-acid reaction and is as follows: 10 cc. of the urine are treated in a graduated flask of 500 cc. capacity with 15 cc. of a 1.2 per cent solution of picric acid and 5 cc. of a 10 per cent NaOH solution. After shaking and allowing to stand for five minutes it is diluted with water to 500 cc. and mixed. This solution is now compared in a DUBOSCQ colorimeter with a 1/2 normal potassium-dicromate solution. The latter solution has in a layer 8 mm. thick exactly the same intensity of color as a layer 8.1 mm. thick of a solution of 10 milligrams creatinine after the addition of 15 cc. picric-acid solution and 5 cc. NaOH solution and dilution to 500 cc. The calculations are simple. For example, in case the urine tested in a layer 7.2 mm. thick has the same color as the dichromate solution in a layer 8 mm. thick, then the quantity of creatinine in 10 cc. of the urine will be $=\frac{8.1}{7.2}\times 10$, or 11.25 milligrams. This method has been tried by many authorities and found to be trustworthy.

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[1] Zeitschr. f. physiol. Chem., **41**.

[2] Folin and Blanck, Journ. of biol. Chem., **8**, with Denis, *ibid.*, **8**.

[3] Zeitschr. f. physiol. Chem., **10** and **14**.

[4] *Ibid.*, **41**.

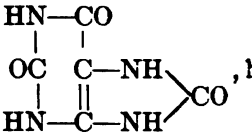
The same method is used in the determination of creatine, which for this purpose is first converted into creatinine by warming with dilute mineral acid. The quantity of creatine is the difference obtained between the values for creatinine before and after treatment with acid. More detailed directions can be found in the cited works of FOLIN, v. HOOGENHUYZE and VERPLOEGH, GOTTLIEB and STANGASSINGER.

In regard to other methods, see the works of KOLISCH and GREGOR.[1]

Xanthocreatinine, $C_8H_{10}N_4O$. This body, which was first prepared from meat extract by GAUTIER, has been found, by MONARI, in dog's urine after the injection of creatinine into the abdominal cavity, and in human urine after several hours of exhaustive marching. According to COLASANTI it occurs to a relatively greater extent in lion's urine. STADTHAGEN[2] considers the xanthocreatinine isolated from human urine after strenuous muscular activity as impure creatinine.

Xanthocreatinine forms thin sulphur-yellow plates, similar to cholesterin, which have a bitter taste. It dissolves in cold water and in alcohol, and gives a crystalline compound with hydrochloric acid and a double compound with gold and platinum chloride. It gives a compound with zinc chloride, which crystallizes in fine needles. Xanthocreatinine has a poisonous action.

Methylguanidine occurs, according to ACHELIS, KUTSCHER and LOHMANN, to a slight extent as a regular constituent of the urine of man, horse and dog. It has been found in urines associated with *dimethylguanidine* by ENGELAND.[3]

Uric Acid, $\overline{Ur}$, $C_5H_4N_4O_3$; 2, 6, 8-trioxypurine,  , has been prepared synthetically by HORBACZEWSKI by fusing urea and glycocoll, or by heating trichlorlactic-acid amide with an excess of urea. BEHREND and ROOSEN prepared it from isodialuric acid and urea; it is also readily produced from isouric acid on boiling with hydrochloric acid (E. FISCHER and TÜLLNER) and finally E. FISCHER and ACH[4] have prepared uric acid from pseudouric acid by heating with oxalic acid to 145° C.

On strongly heating uric acid it decomposes with the formation of *urea*, *hydrocyanic acid*, *cyanuric acid*, and *ammonia*. On heating with concentrated hydrochloric acid in sealed tubes to 170° C. it splits into *glycocoll*, *carbon dioxide*, and *ammonia*. By the action of oxidising agents splitting and oxidation take place, and either monoureides or diureides are produced. By oxidation with lead peroxide, *carbon dioxide*,

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[1] Kolisch, Centralbl. f. innere Med., 1895; Gregor, Zeitschr. f. physiol. Chem., **31**.

[2] Gautier, Bull. de l'acad. de méd. (2), **15**, and Bull. de la soc. chim. (2), **48**; Monari, Maly's Jahresber., **17**; Colasanti, Arch. ital. d. Biologie, **15**, Fasc. 3; Stadthagen, Zeitschr. f. klin. Med., **15**.

[3] Achelis, Centralbl. f. Physiol., **20**, 455, and Zeitschr. f. physiol. Chem., **50**; Kutscher and Lohmann, *ibid.*, **49**; Engeland, *ibid.*, **57**.

[4] Horbaczewski, Monatshefte f. Chem., **6** and 8; Behrend and Roosen, Ber. d. d. chem. Gesellsch., **21**; Fischer and Tüllner, *ibid.*, **35**; Fischer and Ach, *ibid.*, **28**.

oxalic acid, *urea*, and *allantoin*, which last is glyoxyldiureide, are produced (see below). By oxidation with nitric acid in the cold, *urea* and a monoureide, the mesoxalyl urea, or *alloxan*, are obtained, $C_5H_4N_4O_3+O+H_2O=C_4H_2N_2O_4+(NH_2)_2CO$. On warming with nitric acid, alloxan yields carbon dioxide and oxalyl urea, or *parabanic acid*, $C_3H_2N_2O_3$. By the addition of water the parabanic acid passes into *oxaluric acid*, $C_3H_4N_2O_4$, traces of which are found in the urine and which easily splits into oxalic acid and urea. In alkaline solution uric acid may, by taking up water and oxygen, be transformed into a new acid, uroxanic acid, $C_5H_8N_4O_6$, which may then be changed into oxonic acid, $C_4H_5N_3O_4$.[1] On the oxidation of uric acid by hydrogen peroxide in alkaline solution SCHITTENHELM and WIENER[2] have obtained urea with carbonyl diurea as intermediary product. Uric acid may, as F. and L. SESTINI as well as GERARD have shown, undergo bacterial fermentation with the formation of urea. According to ULPIANI and CINGOLANI,[3] uric acid is quantitatively split into urea and carbon dioxide, according to the equation

$$C_5H_4N_4O_3+2H_2O+3O=3CO_2+2CO(NH_2)_2.$$

Uric acid occurs most abundantly in the urine of birds and of scaly amphibians, in which animals the greater part of the nitrogen of the urine appears in this form. Uric acid frequently occurs in the urine of carnivorous mammalia, but is sometimes absent; in urine of herbivora it is habitually present, though only as traces; in human urine it occurs in greater but still small and variable amounts. Traces of uric acid are also found in several organs and tissues, as in the spleen, lungs, heart, pancreas, liver (especially in birds), and in the brain. It always occurs in the blood of birds. Traces have been found in human blood under normal conditions. Under pathological conditions it occurs to an increased extent in the blood, as in pneumonia and nephritis, but especially in leucæmia and sometimes also in arthritis. Uric acid also occurs in large quantities in "chalk-stones," certain urinary calculi, and in guano. It has also been detected in the urine of insects and certain snails, as also in the wings (which it colors white) of certain butterflies (HOPKINS[4]).

The amount of uric acid eliminated with human urine is subject to considerable individual variation, but amounts on an average to 0.7

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[1] See Sundwik, Zeitschr. f. physiol. Chem., **20** and **41**; also Behrend, Annal. d. Chem. u. Pharm., **233**.

[2] Zeitschr. f. physiol. Chem., **62**.

[3] See Chem. Centralbl., 1903, where the other investigators are cited, and Centralbl. f. Physiol., **19**.

[4] Philos. Trans. Roy. Soc., **186**, B, 661.

gram per day on a mixed diet. The ratio of uric acid to urea varies considerably with a mixed diet, but is on an average 1:50–1:70. In newborn infants and in the first days of life the elimination of uric acid is relatively increased, and the relation between uric acid and urea has been found to be 1:6.42–17.1.

We used to ascribe an increasing action upon the elimination of uric acid to protein food, but the investigations of HIRSCHFELD, ROSENFELD and ORGLER, SIVÉN, BURIAN, and SHUR,[1] and many others have positively proven that a diet rich in protein does not itself increase the elimination of uric acid, but only according to the amount of nucleins or purine bodies contained therein. The common assumption that the elimination of uric acid is smaller with a vegetable diet than with an animal diet, when the quantity may be 2 grams or more per twenty-four hours, is explained by this.[2]

Still a purine-free diet is not without some influence upon the elimination of uric acid, as the quantity of uric acid eliminated with a purine-free diet is considerably greater than in starvation and can be increased by protein feeding. The action of the food-protein is here probably an indirect one, consisting in that the proteins raise the work of the digestive glands and the metabolism of their cells and thereby also raise the endogenous uric acid formation (see below) somewhat.[3] Work and rest do not seem to have any special influence upon the uric acid elimination, although according to the confirmed statement of SIVÉN and LEATHES[4] the elimination in the night is less than in the morning hours.

The reports in regard to the influence of other circumstances, as well as of different substances, on the elimination of uric acid are diverse. This is in part due to the fact that the earlier investigators used an inaccurate method (HEINTZ), and also that the extent of uric-acid elimination is dependent in the first place upon the individuality. Thus the investigators are not in accord in regard to the action of drinking-

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[1] See the extensive review of the literature in Wiener, "Die Harnsäure," in Ergebnisse der Physiologie, **1**, Abt. 1, 1902.

[2] J. Ranke, Beobachtungen und Versuche über die Ausscheidung der Harnsäure, etc. (München, 1858); Mareš, Centralbl. f. d. med. Wissensch., 1888; Horbaczewski, Wien. Sitzungsber., **100**, Abt. 3, 1891. In regard to the action of various diets the reader is referred to the above-cited authors, and especially to A. Hermann, Arch. f. klin. Med., **43**, and Camerer, Zeitschr. f. Biologie, **33**, and Folin, Amer. Journ. of Physiol., **13**.

[3] See Hirschstein Arch. f. exp. Path. u. Pharm., **57**; Smétanka, Pflüger's Arch., **138** and **149**; Mareš, *ibid.*, **134** and **149**. Contrary views, Brugsch and Schittenhelm, Zeitschr. f. exp. Path. u. Therp., **4**, and Sivén, Pflüger's Arch., **146**.

[4] Sivén, Skand. Arch. f. Physiol., **11**; Leathes, Journ. of Physiol, **35**; see also Kennaway, Journ. of Physiol., **38**.

water[1] and of alkalies.[2] Certain medicines, such as quinine and atropine, diminish, while others, such as pilocarpine and, as it seems, salicylic acid,[3] increase the elimination of uric acid.

There is much diversity of opinion regarding the elimination of uric acid in disease,[4] although it is known that it is increased after an abundant destruction of nucleated cells as in pneumonia, after the crisis, and in leucæmia. In the latter in most cases not only is the elimination to the urea increased absolutely, but also relatively; and the relation between uric acid and urea (total nitrogen calculated as urea) may in lineal leucæmia even be 1:9, while under normal conditions, according to different investigators, it is 1:50 to 70 to 100. As to the behavior of uric acid in gout, authorities are by no means agreed. That the blood contains uric acid in gout has been repeatedly shown, and it is also found in this disease with a purine-free diet (BRUGSCH and SCHITTENHELM). According to these investigators a diminished enzymotic decomposition of uric acid occurs in the body in gout and this causes the occurrence of uric acid in the blood and its accumulation in certain tissues. Strong arguments against this view have been presented by others such as WELLS and CORPER, MILLER and JONES.[5]

Formation of Uric Acid in the Organism. Since HORBACZEWSKI first showed that uric acid could be produced by oxidation from the nuclein-rich spleen-pulp or nucleins outside of the body, he also showed that nucleins when introduced into the animal body caused an increase in the elimination of uric acid. These observations have been confirmed, and at the same time developed by the work of a great number of investigators, and we are sure that uric acid can be produced from purine bases either outside or inside the animal body, and also that food rich in nucleins (especially the thymus gland) increases the elimination of uric acid. It is nevertheless true that a few investigators after introducing pure purine bases into the organism could not observe any essential rise in the uric acid or its transformation products; still we have a large number of recent investigations which positively show that nucleic acids, as well as purine bases, when introduced into the animal body are transformed in abundant quantities into uric acid in the body.[6] At present

[1] See Schöndorff, Pflüger's Arch., **46**, which contains the pertinent literature.

[2] See Clar, Centralbl. f. d. med. Wissensch., 1888; Haig, Journ. of Physiol., 8; and A. Hermann, Arch. f. klin. Med., **43**.

[3] See Bohland, cited from Maly's Jahresber., **26**; Schreiber and Zaudy, *ibid.*, **30**.

[4] In regard to the extensive literature on the elimination of uric acid in disease we must refer to special works on internal diseases.

[5] Brugsch and Schittenhelm, Zeitschr. f. exp. Path. u. Therp., 4; Wells and Corper, Journ. of biol. Chem., 6; Miller and Jones, Zeitschr. f. physiol. Chem., **61**.

[6] As it is not within the scope of this book to enter into a discussion of the numerous researches on this subject, we will refer to Wiener, "Die Harnsäure," Ergebnisse

we consider the formation of uric acid from the purine bases of the nuclein substances as a positively proven fact.

According to the original view of HORBACZEWSKI the nucleins do not directly (by their purine bases) cause an increased elimination of uric acid, but indirectly by causing a leucocytosis with a consequent destruction of leucocytes. This view has been justly discarded on account of the above-mentioned conditions; still on the other hand it cannot be denied that the formation of uric acid is also in certain regards related to the formation or the destruction of leucocytes and to the metabolism in the cells as a whole.[1]

The uric acid, in so far as it is produced from nuclein bases, is in part derived from the nucleins of the destroyed cells of the body and in part from the nucleins or free purine bases introduced with the food. It is therefore possible to admit, with BURIAN and SCHUR,[2] of a double origin for the uric acid as well as the urinary purines (all purine bodies of the urine, including the uric acid), namely, an *endogenous* and an *exogenous* origin. BURIAN and SCHUR attempted to determine the quantity of endogenous urinary purines by feeding with sufficient food, but as free as possible from purine bodies, and they found that this quantity was constant for every individual, while it was variable for different persons. The observations of many other investigators have led to similar conclusions, and we are now unanimous in our opinion that the uric acid originating from the nucleins is partly endogenous and partly exogenous, and that the amount of endogenous uric acid is only very slightly dependent upon the protein content of the food.

The formation of uric acid from the nucleins or the purine bases seems at least in great part to be of an enzymotic kind. After it was shown that certain organs, such as the liver and spleen, had the power of converting oxypurines into uric acid in the presence of oxygen (HORBACZEWSKI, SPITZER and WIENER[3]), recently SCHITTENHELM, BURIAN, JONES and co-workers[4], by more careful investigations have shown that enzymes

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der Physiol., **1**, Abt. 1, 1902. See also Schittenhelm, Zeitschr. f. physiol. Chem., **62**, with Frank, *ibid.*, **63**, with Seisser, Zeitschr. f. exp. Path. u. Ther., **7**; Abderhalden, London and Schittenhelm, Zeitschr. f. physiol. Chem., **61**; Mendel and Lyman., Journ. of biol. Chem., **8**.

[1] See Plimmer, Dick and Lieb, Journ. of Physiol., **39**; Mareš Pflüger's Arch., **134**, and Smétanka, *ibid.*, **138**.

[2] Pflüger's Arch., **80**, **87**, and **94**.

[3] See footnote, 6, page 701.

[4] Schittenhelm, Zeitschr. f. physiol. Chem., **42**, **43**, **45**, **46**, **57**, **62**, **66**, with Schmid, *ibid.*, **50**, and Zeitschr. f. exp. Path. u. Therap., **4**; Burian, Zeitschr. f. physiol. Chem., **43**; Jones and Partridge, *ibid.*, **42**; Jones with Winternitz, *ibid.*, **44** and **60**; Jones, *ibid.*, **45**, **65**, with Austrian, *ibid.*, **48**, with Miller, *ibid.*, **61**; Jones, Journ. of biol. Chem., **9**; Wells, *ibid.*, **7**; Mendel and Mitchell, Amer. Journ. of Physiol., **20**.

of different kinds act together. By means of the two deamidizing enzymes *adenase* and *guanase*, the adenine and guanine are transformed into hypoxanthine and xanthine respectively, and from the latter by means of an oxidizing enzyme, called *xanthine oxidase* by BURIAN, the uric acid is formed. In the formation of uric acid from the nucleoproteins we must admit of a gradual decomposition of these by the aid of different enzymes, proteases, nucleases and deamidases. The deamidases seem to be present in most organs, and we have numerous investigations upon their distribution, especially those of JONES and SCHITTENHELM and his collaborators.[1] The distribution is not the same in all animals and the reports regarding it are unfortunately conflicting (SCHITTENHELM, JONES and MILLER). We must exercise the greatest caution in drawing conclusions as to the occurrence of these enzymes, and from experiments made with the extracts of organs, because it seems as if also other unknown factors must be considered in the formation of uric acid. Thus JONES has with ROHDE [2] shown that in rats the organs do not contain any xanthine oxidase, and that nevertheless the urine of this animal contains uric acid. On the other hand deamidases occur in the organs of monkeys (and xanthine oxidase in the liver) but the urine does not contain any uric acid and only traces of allantoin (WELLS).[3] The possibility of a uric acid formation in man and mammalia in another way from the enzymotic destruction of the purines cannot, for several reasons, be denied.

In birds the conditions are different. v. MACH [4] has shown that in the bird family a part of the uric acid may be formed from the purine bodies. The chief quantity of uric acid, however, is undoubtedly formed in birds by synthesis.

The formation of uric acid in birds is increased by the administration of ammonium salts (v. SCHRÖDER), and urea acts in a similar manner (MEYER and JAFFÉ). MINKOWSKI observed, in geese with extirpated livers, a very significant decrease in the elimination of uric acid, while the elimination of ammonia was increased to a corresponding degree. This indicates a participation of ammonia in the formation of uric acid in the organism of birds; and as MINKOWSKI has also found, after the extirpation of the liver, that considerable amounts of lactic acid occur in the urine, it is probable that the uric acid in birds is produced in the liver by synthesis, perhaps from lactic acid and ammonia;

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[1] See footnote 4, page 703.

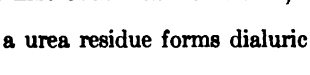
[2] Jones, Zeitschr. f. physiol. Chem., **65**, with Alice Rohde, Journ. of biol. Chem., **7**; see also Voegtlin and Jones, Zeitschr. f. physiol. Chem., **66**.

[3] Journ. of biol. Chem., **7**.

[4] Arch. f. exp. Path. u. Pharm., **24**.

although, as SALASKIN and ZALESKI and LANG have shown, after the extirpation of the liver, and increase in the formation of lactic acid primarily occurs, and this causes an increase in the elimination of ammonia (neutralization ammonia). The direct proof for the uric-acid formation from ammonia and lactic acid in the liver of birds has been given by KOWALEWSKY and SALASKIN[1] by means of blood-transfusion experiments on geese with extirpated livers. They observed a relatively abundant formation of uric acid after the addition of ammonium lactate and a still greater formation after arginine. They not only consider ammonium lactate but also amino-acids as substances from which the uric acid can be produced in the liver by synthesis. That these, for example, leucine, glycocoll, and aspartic acid, increase the elimination of uric acid in birds was first shown by v. KNIERIEM.[2]

The possibility of a formation of uric acid from lactic acid has been shown in another manner by WIENER,[3] namely, by feeding birds with urea and lactic acid and different non-nitrogenous substances, oxy-, keto-, and dibasic acids of the aliphatic series. The dibasic acids, with a chain of 3 carbon atoms or their ureides, showed themselves most active as uric-acid formers, and WIENER is therefore of the opinion that the active substances must first be converted into dibasic acids. By the attachment of a urea residue the corresponding ureide is produced, according to WIENER, and from this the uric acid is derived by the attachment of a second urea residue.

Among the substances tested, only tartronic acid and its ureide, dialuric acid, have shown themselves active in the experiments with the isolated organs, and WIENER therefore also considers that the other acids must be first converted into tartronic acid by oxidation or reduction. From lactic acid, $CH_3.CH(OH).COOH$, we first obtain tartronic acid, $COOH.CH(OH).COOH$, which by the attachment of a urea residue forms dialuric acid,  , and from this, by the attachment of a second urea residue, uric acid is formed.

Recently IZAR[4] has shown on perfusing blood containing urea and dialuric acid through the liver of a dog and at the same time saturating the blood with carbon dioxide, that an abundant formation of uric acid occurred, and that a combined action between an enzyme occurring

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[1] v. Schröder, Zeitschr. f. physiol. Chem., **2**; Meyer and Jaffé, Ber. d. f. Chem. Gesellsch., **10**; Minkowski, Arch. f. exp. Path. u. Pharm., **21** and **31**; Salaskin and Zaleski, Zeitschr. f. physiol. Chem., **29**; Lang, *ibid.*, **32**; Kowalewsky and Salaskin, *ibid.*, **33**.

[2] Zeitschr. f. Biologie, **13**.

[3] Hofmeister's Beiträge, **2**. See also Arch. f. exp. Path. u. Pharm., **42**, and Ergebnisse d. Physiol., **1**, Abt. 1, 1902.

[4] Zeitschr. f. physiol. Chem., **73**, see also *ibid.*, **65**.

in the blood and an alcohol-soluble co-enzyme occurring in the liver and spleen took place. He has besides this also given further proof of the formation of uric acid in the bird-liver from urea and ammonium carbonate.

We cannot give any positive answer as to the question whether uric acid is formed by synthesis in man and other mammalia. WIENER has reported experiments which seem to indicate a synthetic uric-acid formation in the isolated mammalian liver, and he has also obtained an increase in the uric-acid elimination, although only a slight one, after feeding lactic acid and dialuric acid to man. In opposition to these experiments PFEIFFER[1] could find no increase in the elimination of uric acid after feeding malonamide and tartronamide to monkeys as well as tartronic acid and pseudouric acid to monkeys or human beings, and he finds that a synthesis of uric acid in mammalia and man is very doubtful. According to BURIAN[2] we have for the present no proof of a synthetical formation of uric acid in the mammalian liver; in view of the above-mentioned experiments of IZAR, we cannot deny the possibility of a synthetical formation of uric acid also in mammalia and man even if we do not know to what extent this occurs.

The liver seems to be the organ in birds where the synthetical formation of uric acid occurs, and the fact that it was possible for MINKOWSKI[3] to arrest the uric-acid formation by the extirpation of the liver, apparently shows that the liver is the only organ taking part in this synthesis. If a synthesis of uric acid also occurs in man and other mammalia, we must consider the liver as at least one of the organs taking part in the work, as shown by WIENER's and IZAR's investigations. The liver is considered as the most important organ in the oxidative formation of uric acid from nucleins and purine bases. That this organ, at least in the dog, is not the only or at least not the most important follows from the investigations of ABDERHALDEN, LONDON and SCHITTENHELM[4] on dogs with Eck fistula. They found that, on excluding the liver in this manner, that the transformation of the nucleic acid fed, the deamidation of the purine bases and the oxidation of these into uric acid and allantoin was undisturbed. In the dog also other organs must be considered in this connection. It is not known how other animals behave in this regard.

Uric acid when introduced into the mammalian organism is, as first shown by WÖHLER and FRERICHS, in the dog, and later substantiated

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[1] Hofmeister's Beiträge, **10**.
[2] Zeitschr. f. physiol. Chem., **43**.
[3] l. c.
[4] Zeitschr. f. physiol. Chem., **61**.

by several experimenters,[1] in great part destroyed and more or less completely changed into urea. As shown by WÖHLER and FRERICHS for the dog and by later investigators[2] also for cats, rabbits and other animals, that allantoin is the most essential or indeed the chief decomposition product is now considered as positively proven. In man, on the contrary, the conditions are different. According to WIECHOWSKI[3] probably also a formation of allantoin from uric acid takes place in man, but it is only of such an extent as to be without consideration, while in the dog for example about 96 per cent of the purine base nitrogen may appear as allantoin in the urine. According to the investigations of FRANK and SCHITTENHELM[4] the uric acid in man is in part transformed into urea.

This different behavior of uric acid in the metabolism of man and animals depends, as numerous investigations[5] have shown, upon the occurrence of a urocolytic enzyme in the liver and also other organs of animals, which transforms the uric acid into allantoin with the taking up oxygen and splitting off of carbon dioxide. This enzyme, which has been called *uricolase* and also *uricase* and whose occurrence in the organs of different animals varies, is absent in the organs of man. The results obtained in regard to the enzymotic transformation of uric acid by experiments with organ extracts must be judged with the greatest care. Thus according to the statements of WIECHOWSKI, BATTELLI and STERN and SCHITTENHELM,[6] in dogs, the liver is the only organ which in a test-tube shows a positive uricolysis; still in dogs, with excluded livers (Eck fistula) such an abundant formation of allantoin from uric acid occurs so that only 10–20 per cent of the uric acid escapes this transformation.[7]

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[1] Wöhler and Frerichs, Annal. d. Chem. u. Pharm., **65**. See also Wiener, Ergebnisse der Physiologie, **1**, Abt. 1.

[2] Salkowski, Zeitschr. f. physiol. Chem., **25**, and Ber. d. d. Chem. Gesellsch., **9**; Mendel and Brown, Amer. Jour. of Physiol., **3**; Mendel and White, *ibid.*, **12**; Wiechowski, Arch. f. exp. Path. u. Pharm., **60**, and Bioch. Zeitschr., **19** and **25**, with Wiener, Hofmeister's Beiträge, **9**; Schittenhelm, Zeitschr. f. physiol. Chem., **62**, with Seisser, Zeitschr. f. exp. Path. v. Ther., **7**; Abderhalden, London and Schittenhelm, Zeitschr. f. physiol. Chem., **61**.

[3] Bioch. Zeitschr., **25**.

[4] Zeitschr. f. physiol. Chem., **63**.

[5] Chassevant and Richet, Comp. rend. soc. biolog., **49**; Ascoli, Pflüger's Arch., **72**; Jacoby, Virchow's Arch., **157**; Wiener, Arch. f. exp. Path. u. Pharm., **42**, and Centralbl. f. Physiol., **18**; Schittenhelm, Zeitschr. f. physiol. Chem., **43**, **45**, and **63**; Burian, *ibid.*, **43**; Almagia, Hofmeister's Beiträge, **7**; Pfeiffer, *ibid.*, **7**; Wiechowski and Wiener, *ibid.*, **9**; Galeotti, Bioch. Zeitschr., **20**; Battelli and Stern, *ibid.*, **19**; Scaffidi, *ibid.*, **18**; Miller and Jones, Zeitschr. f. physiol. Chem., **61**; Wells, Journ. of biol. Chem., **7**, with Corper, *ibid.*, **6**.

[6] See Schittenhelm, Zeitschr. f. physiol. Chem., **63**, 256.

[7] Abderhalden, London and Schittenhelm, l. c.

ASCOLI, IZAR, BEZZOLA and PRETI [1] have studied the remarkable ability of the liver of destroying uric acid in the blood by transfusing the arterial blood through this organ and on transfusing the blood, saturated with CO_2, they have regenerated the uric acid. It is not known what becomes of the uric acid in these cases and from what substance the regeneration occurs. PRETI has shown that in the regeneration a combined action of an enzyme in the blood with a co-enzyme of the liver, takes place.

From this power of the various organs of destroying uric acid it follows that the quantity of uric acid eliminated is not a sure indication of the amount of the acid formed. We must, therefore, admit that a part of the uric acid formed in the body is destroyed in a manner similar to that introduced from without. BURIAN and SCHUR [2] have indeed suggested a factor, the so-called "integral factor," with which the quantity of uric acid eliminated in the twenty-four hours must be multiplied in order to find the quantity of uric acid formed during this time. Such calculations are necessarily very uncertain and are for the present not admissible.

Properties and Reactions of Uric Acid. Pure uric acid is a white, odorless, and tasteless powder consisting of very small rhombic prisms or plates. Impure uric acid is easily obtained as somewhat larger, colored crystals.

In rapid crystallization, small, thin, four-sided, apparently colorless, rhombic prisms are formed, which can be seen only by the aid of the microscope, and these sometimes appear as spools because of the rounding of their obtuse angles. The plates are sometimes six-sided, irregularly developed; in other cases they are rectangular with partly straight and partly jagged sides; and in other cases they show still more irregular forms, the so-called dumb-bells, etc. In slow crystallization, as when the urine deposits a sediment or when treated with acid, large, invariably colored crystals separate. Examined with the microscope these crystals always appear yellow or yellowish brown in color. The most common type is the whetstone shape, formed by the rounding off of the obtuse angles of the rhombic plate. The whetstones are generally connected, two or more crossing each other. Besides these forms, rosettes of prismatic crystals, irregular crosses, brown-colored rough masses of broken-up crystals and prisms occur, as well as other forms.

Uric acid is insoluble in alcohol and ether; it is rather easily soluble in boiling glycerin, but very insoluble in cold water, in 39480 parts at 18° C. (HIS and PAUL), and in 15505 parts at 37° (GUDZENT). At this temperature, according to HIS and PAUL, 9.5 per cent of the uric acid is dissociated in the saturated solution. Because of the reduction

[1] See Zeitschr. f. physiol. Chem., 58, 62, 64 and 65.

[2] Pflüger's Arch., 87.

in the dissociation on the addition of strong acids, uric acid is soluble with difficulty in the presence of mineral acids. It is soluble in a warm solution of sodium diphosphate, and in the presence of an excess of uric acid, monophosphate and acid urate are produced. It is ordinarily assumed that sodium diphosphate forms a solvent for the uric acid in the urine, while according to GUDZENT this is not dissolved by the monophosphate. RÜDEL [1] believes that urea is an important solvent, but this view has not been confirmed by the observations of HIS and PAUL. Uric acid is not only dissolved by alkalies and alkali carbonates, but also by several organic bases, such as ethylamine and propylamine, piperidine and piperazine. Uric acid can form supersaturated solutions with alkalies and these, according to SCHADE and BODEN [2] contain colloidal uric acid and they may gelatinize on cooling as well as under other conditions. Uric acid dissolves, without decomposing, in concentrated sulphuric acid. It is completely precipitated from the urine by picric acid (JAFFÉ [3]). Uric acid gives a chocolate-brown precipitate with phosphotungstic acid in the presence of hydrochloric acid.[4]

Uric acid is dibasic and consequently forms two series of salts, neutral and acid. Of the alkali urates the lithium salts are the most soluble and the acid ammonium salt is the most insoluble. The acid alkali urates are very insoluble and separate as a sediment (*sedimentum lateritium*) from concentrated urine on cooling. According to GUDZENT 1 liter of water at 18° C. dissolves (as primary salts) 1.5313 grams potassium, 0.8328 gram sodium, and 0.4141 gram ammonium urate, and at 37° C. 2.7002, 1.5043 and 0.7413 grams of the respective urates.[5] The salts of the alkaline earths are soluble with great difficulty. The above solubilities apply only, in GUDZENT'S [6] experience, to the freshly prepared solution, as the solubility to a certain limit gradually diminishes, due to intramolecular transposition (change of the uric acid from the lactam-form into the lactim-form).

Besides the mono- and diurates also "quadriurates" have been described and these occur in the excrement of snakes and birds and in the *sedimentum*

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[1] His, Jr., and Paul, Zeitschr. f. physiol. Chem., **31**; Smale, Centralbl. f. physiol., **9**; Rüdel, Arch. f. exp. Path. u. Pharm., **30**; Gudzent, Zeitschr. f. physiol. Chem., **60** and **63**.

[2] Zeitschr. f. physiol. Chem., **88**.

[3] *Ibid.*, **10**.

[4] In regard to the combinations of formaldehyde and uric acid, see Nicolaier, Deutsch. Arch. f. klin. Med., **89** (1906).

[5] Determinations of the solubility of the monourates in serum have been made by Gudzent, Zeitschr. f. physiol. Chem., **63**. See also Bechhold and Ziegler, Bioch. Zeitschr., **20**.

[6] Zeitschr. f. physiol. Chem., **56** and **60**.

lateritium. Whether these quadriurates, which have recently been studied by RINGER, KOHLER and SCHMUTZER,[1] are chemical combinations of 2 molecules uric acid and 1 atom of K or Na or are mixtures, so-called solid solutions of uric acid in monourates, is still a disputed question.

If a little uric acid in substance is treated on a porcelain dish with a few drops of nitric acid, the uric acid dissolves on warming, with a strong development of gas, and after thoroughly drying on the water-bath a beautiful red residue is obtained, which turns a purple-red (ammonium purpurate or murexide) on the addition of a little ammonia. If instead of the ammonia we add a little caustic soda (after cooling), the color becomes deeper blue or bluish violet. This color disappears quickly on warming, differing from certain purine bodies. This reaction is called the *murexide test*.

A solution of phosphotungstic acid, prepared according to certain directions, gives with a solution of uric acid, when treated with an excess of sodium carbonate, a beautiful blue solution. This extremely delicate reaction (1:500,000) was suggested by FOLIN and DENIS.[2]

Uric acid does not reduce an alkaline solution of bismuth, while, on the contrary, it reduces an alkaline cupric-hydroxide solution. In the presence of only a little copper salt we obtain a white precipitate consisting of cuprous urate. In the presence of more copper salt red cuprous oxide separates. The compound of uric acid with cuprous oxide is formed when copper salts are reduced by glucose or a bisulphite in alkaline solution in the presence of a sufficient amount of urate.

If a solution of uric acid in water containing alkali carbonate is treated with magnesium mixture and then a silver-nitrate solution added, a gelatinous precipitate of silver-magnesium urate is formed. If a drop of uric acid dissolved in sodium carbonate is placed on a piece of filter-paper which has been previously treated with silver-nitrate solution, a reduction of silver oxide occurs, producing a brownish-black or, in the presence of only 0.002 milligram of uric acid, a yellow spot (SCHIFF'S test).

If a weak alkaline solution of uric acid in water is treated with a soluble zinc salt, a white precipitate is produced, which on the filter in the presence of alkali is oxidized by the air, and becomes sky-blue in color, especially in sunlight. Potassium persulphate causes a blue coloration immediately (GANASSINI'S reaction [3]).

The precipitation of free uric acid from its alkali salts by means of acids can be prevented to some extent by the presence of thymic acid or nucleic acid (GOTO). According to SEO we are here dealing with combinations of 1 molecule nucleic

[1] Ringer, *ibid.*, 67 (literature) and 75; Kohler, *ibid.*, 70 and 72; Ringer and Schmutzer, *ibid.*, 82.

[2] Journ. of biol. Chem., 12.

[3] Cited f. Bioch. Centralbl., 8, 250.

acid and 2 molecules uric acid, which protects the uric acid within the body against destruction or transformation into allantoin. This view is incorrect, according to SCHITTENHELM and SEISSER.[1] According to them no constant combination between nucleic acid and uric acid exists, and in rabbits the nucleic acid does not protect the uric acid from transformation to allantoin.

Preparation of Uric Acid from Urine. Filtered normal urine is treated with 20–30 cc. of 25-per cent hydrochloric acid for each liter of urine. After forty-eight hours collect the crystals and purify them by redissolving in dilute alkali, decolorizing with animal charcoal and reprecipitating with hydrochloric acid. Large quantities of uric acid are easily obtained from the excrement of serpents by boiling it with dilute caustic potash (5-per cent) until no more ammonia is developed. A current of carbon dioxide is passed through the filtrate until it barely has an alkaline reaction; dissolve the separated and washed acid potassium urate in caustic potash, and precipitate the uric acid in the filtrate by addition of an excess of hydrochloric acid.

Quantitative Estimation of Uric Acid in the Urine. As the older method suggested by HEINTZ, even after recent modifications, gives inaccurate results, it will not be considered here.

Salkowski and Ludwig's[2] method consists in precipitating the uric acid, by silver nitrate, from the urine previously treated with magnesium mixture, and weighing the uric acid obtained from the silver precipitate. Uric acid determinations by this method are often performed according to the suggestion of E. LUDWIG, which requires the following solutions:

1. An AMMONIACAL SILVER-NITRATE SOLUTION, which contains in 1 liter 26 grams of silver nitrate and a quantity of ammonia sufficient to redissolve completely the precipitate produced by the first addition of ammonia. 2. MAGNESIA MIXTURE. Dissolve 100 grams of crystallized magnesium chloride in water, add ammonia until the liquid smells strongly of it, and enough ammonium chloride to dissolve the precipitate; then dilute the solution to 1 liter. 3. SODIUM SULPHIDE SOLUTION. Dissolve 10 grams of caustic soda which is free from nitric acid and nitrous acid in 1 liter of water. One half of this solution is completely saturated with sulphuretted hydrogen and then mixed with the other half.

The concentration of the three solutions is so arranged that 10 cc. of each is sufficient for 100 cc. of the urine.

100–200 cc., according to concentration, of the filtered urine, freed from protein (by boiling after the addition of a few drops of acetic acid), are poured into a beaker. In another vessel mix 10–20 cc. of the silver solution with 10–20 cc. of the magnesia mixture and add ammonia, and when necessary also some ammonium chloride, until the mixture is clear. This solution is added to the urine while stirring, and the mixture allowed to stand quietly for half an hour. The precipitate is collected on a filter, washed with ammoniacal water, and then returned to

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[1] Goto, Zeitschr. f. physiol. Chem., **30**; Seo, Arch. f. exp. Path. u. Pharm., **58**; Schittenhelm and Seisser, Zeitschr. f. exp. Path. u. Ther., **7**.

[2] Salkowski, Virchow's Arch., **52**; Pflüger's Arch., **5**; Salkowski, Laboratory Manual of Physiol. and Path. Chem., translated by Orndorff, 1904; Ludwig, Wien. med. Jahrbuch, 1884, and Zeitschr. f. anal Chem., **24**.

the same beaker by the aid of a glass rod and a wash-bottle, without destroying the filter. Now heat to boiling 10–20 cc. of the alkali-sulphide solution, which has previously been diluted with an equal volume of water, and allow this solution to flow through the above filter into the beaker containing the silver precipitate; wash with boiling water, and warm the contents of the beaker on a water-bath for a time, stirring constantly. After cooling, filter into a porcelain dish, wash the filter with boiling water, acidify the filtrate with hydrochloric acid, evaporate it to about 15 cc., add a few drops more of hydrochloric acid, and allow it to stand for twenty-four hours. The uric acid which has crystallized is collected on a small weighed filter, washed with water, alcohol, ether, and carbon disulphide, dried at 100–110° C., and weighed. For each 10 cc. of aqueous filtrate we must add 0.00048 gram uric acid to the quantity found directly. Instead of the weighed filter-paper a glass tube filled with glass wool as described in other handbooks may be substituted (LUDWIG). Too intense or too long continued heating with the alkali sulphide must be prevented, otherwise a part of the uric acid may be decomposed.

SALKOWSKI deviates from this procedure by first precipitating the urine with a magnesium mixture (50 cc. to 200 cc. urine), filling up to 300 cc., and filtering. Of the filtrate, 200 cc. are precipitated by 10–15 cc. of a 3-per cent silver-nitrate solution. The silver precipitate is shaken with 200-300 cc. of water acidified with a few drops of hydrochloric acid, decomposed by sulphuretted hydrogen, heated to boiling, the silver-sulphide precipitate boiled with fresh water, filtered, the filtrate concentrated to a few cubic centimeters, treated with 5–8 drops of hydrochloric acid, and allowed to stand until the next day. According to SALKOWSKI and KASHIWABARA [1] the precipitation with zinc salts can also be used in the estimation of uric acid.

Hopkins' method is based on the fact that the uric acid is completely precipitated from the urine as ammonium urate on saturating with ammonium chloride. The uric acid can either be weighed after being set free by hydrochloric acid or it can be determined in several ways—by titration with potassium permanganate or by the KJELDAHL method. Several modifications of this method have been worked out by FOLIN, FOLIN and SCHAFFER, WÖRNER, and JOLLES.[2] Of these methods we shall describe only that suggested by FOLIN-SCHAFFER.

Folin-Schaffer Method. Treat 300 cc. urine with 75 cc. of a solution containing 500 grams of ammonium sulphate, 5 grams of uranium acetate, and 60 cc. of 10 per cent acetic acid in a liter, and filter after five minutes. This removes an unknown constituent of the urine (a protein substance) which would otherwise contaminate the uric acid. Take 125 cc. of the filtrate (corresponding to 100 cc. of the urine) and add 5 cc. of concentrated ammonia. After twenty-four hours the precipitate is filtered off and washed free from chlorine on the filter by means of an ammonium-sulphate solution. The precipitate is washed off the

[1] Zeitschr. f. physiol. Chem., 4.

[2] Hopkins, Journ. of Path, and Bact., 1893, and Proceed. Roy. Soc., 52; Folin, Zeitschr. f. physiol. Chem., 24; Folin and Schaffer, *ibid.*, 32; Wörner, *ibid.*, 29; Jolles, *ibid.*, 29; and Wien. med. Wochenschr., 1903.

filter by water (total 100 cc.) into a flask, treated with 15 cc. of concentrated sulphuric acid, and titrated at 60–63° C. with N/20 potassium-permanganate solution. Each cubic centimeter of this solution corresponds to 3.75 milligrams uric acid. Because of the solubility of the ammonium urate a correction of 3 milligrams must be added for every 100 cc. of the urine.

In regard to the numerous other methods for estimating uric acid, we must refer to special works on the subject, and especially to HUPPERT-NEUBAUER. FOLIN with MACULLUM JR. and with DENIS [1] have suggested a colorimetric method for estimating uric acid, making use of phosphotungstic acid.

Purine Bases (ALLOXURIC BASES). The purine bases found in human urine are *xanthine*, (*guanine*), *hypoxanthine*, *adenine*, *paraxanthine*, *heteroxanthine*, *episarkine*, *epiguanine*, 1-*methylxanthinc*. The occurrence of guanine and carnine (POUCHET) is, according to KRÜGER and SALOMON,[2] not positively shown. The quantity of these bodies in the urine is extremely small and varies in different individuals. FLATOW and REITZENSTEIN [3] found 15.6–45.1 milligrams in the urine voided during twenty-four hours. The quantity of alloxuric bases in the urine is regularly increased after feeding with nucleins or food rich in nucleins, and after an abundant destruction of leucocytes. The quantity is especially increased in leucæmia. We have a number of observations on the elimination of these bodies in different diseases, but they are hardly trustworthy on account of the inaccuracy of the methods used in the determinations. It must also be remarked that the three purine bases, heteroxanthine, paraxanthine, and 1-methylxanthine, which form the chief mass of the purine bases of the urine, are derived, according to numerous investigations [4] from the theobromine, caffeine, and theophylline which occur in the food. With the purine bases we must also differentiate between those of endogenous and those of exogenous origin,[5] and the same factors apply as for the uric acid, viz., the endogenous purine formation represents a value which is somewhat variable for different individuals and relatively

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[1] Journ. of biol. Chem., **13** and **14**.

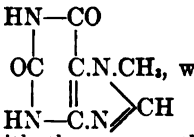
[2] Zeitschr. f. physiol. Chem., **24**; Pouchet, "Contributions à la connaissance des matières extractives de l'urine." Thése, Paris, 1880. Cited from Huppert-Neubauer, 333 and 335.

[3] Deutsch. med. Wochenschr., 1897.

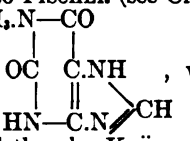
[4] Albanese, Ber. d. d. chem. Gesellsch., **32**; Arch. f. exp. Path. u. Pharm., **35**; Bondzynski and Gottlieb, *ibid.*, **36**, and Ber. d. deutsch. chem. Gesellsch., **28**; E. Fischer, *ibid.*, **30**, 2405; Krüger and Salomon, Zeitschr. f. physiol. Chem., **26**; Krüger and Schmidt, Ber. d. d. chem. Gesellsch., **32**, and Arch. f. exp. Path. u. Pharm., **45**; Kotake, Zeitschr. f. physiol. Chem., **57**.

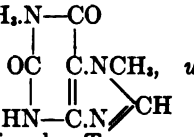
[5] See Burian and Schur, footnote 2, page 702, and Kaufmann and Mohr, Deutsch. Arch. f. klin. Med., **74**.

constant for the same individual. According to SIVÉN,[1] with purine-free diet the elimination of purines is lowest at night and highest in the morning hours. Rest and work do not show any positive difference. As the four true nuclein bases have been treated in Chapter II, it only remains to describe the special urinary purine bodies.

Heteroxanthine, $C_6H_6N_4O_2$, 7-monomethylxanthine,  , was first detected in the urine by SALOMON. It is identical with the monomethylxanthine which passes into the urine after feeding with theobromine or caffeine. SALOMON and NEUBURG [2] found heteroxanthine in the urine of a dog fed entirely upon meat, and this was probably formed by a methylation in the body.

Heteroxanthine crystallizes in shining needles and dissolves with difficulty in cold water (1592 parts at 18° C.). It is readily soluble in ammonia and alkalies. The crystalline sodium salt is insoluble in strong caustic alkali (33-per cent) and dissolves with difficulty in water. The chloride crystallizes beautifully, is relatively insoluble, and is readily decomposed into the free base and hydrochloric acid by water. Heteroxanthine is precipitated by copper sulphate and bisulphite, mercuric chloride, basic lead acetate and ammonia, and by silver nitrate. The silver compound dissolves rather easily in dilute, warm nitric acid; it crystallizes in small rhombic plates or prisms, often grown together, forming characteristic crosses. Heteroxanthine does not give the xanthine reaction, but does give WIEDEL'S reaction, according to FISCHER (see Chapter II).

1-Methylxanthine, $C_6H_6N_4O_2$,  , was first isolated from the urine and studied by KRÜGER, and then by KRÜGER and SALOMON.[3] It is difficultly soluble in cold water, but readily soluble in ammonia and caustic soda, and does not give an insoluble sodium compound. It is readily soluble in dilute acids, and it crystallizes from its acetic-acid solution in thin, generally hexagonal plates. The chloride is decomposed into the base and hydrochloric acid by water. 1-methylxanthine gives crystalline double salts with platinum and gold. It is not precipitated by basic lead acetate, nor when pure by basic lead acetate and ammonia. With ammonia and silver nitrate it gives a gelatinous precipitate. The silver-nitrate compound crystallized from nitric acid forms rosettes of united needles. With the xanthine test with nitric acid it gives an orange coloration on the addition of caustic soda. It gives WEIDEL'S reaction (according to FISCHER) beautifully.

Paraxanthine, $C_7H_8N_4O_2$, 1.7-dimethylxanthine,  , *urotheobromine* (THUDICHUM), was first isolated from the urine by THUDICHUM and

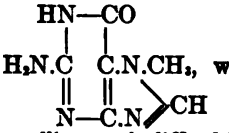
[1] Skand. Arch. f. Physiol., 18.

[2] Salkowski's Festschrift, Berlin, 1904.

[3] Krüger, Arch. f. (Anat. u.) Physiol., 1894; Krüger and Salomon, Zeitschr. f. physiol. Chem., 24.

SALOMON.[1] It crystallizes beautifully in six-sided plates or in needles. The sodium compound crystallizes in rectangular plates or prisms and, like the hetero-xanthine-sodium compound, is insoluble in 33-per cent caustic-soda solution. The sodium compound separates in a crystalline state on neutralizing its solution in water. The chloride is readily soluble and is not decomposed by water. The chloroplatinate crystallizes very beautifully. Mercuric chloride precipitates it only when added in excess and after a long time. The silver-nitrate compound separates as white silky crystals from hot nitric acid on cooling. It gives WEIDEL'S reaction, but not the xanthine test, with nitric acid and alkali.

Episarkine is the name given by BALKE to a purine body occurring in human urine. The same body has been observed by SALOMON [2] in pigs' and dogs' urine, as well as in urine in leucæmia. BALKE gives $C_4H_6N_4O$ as the probable formula for episarkine. It is nearly insoluble in cold water, dissolves with difficulty in hot water, but may be obtained therefrom as long fine needles. Episarkine does not give the xanthine reaction with nitric acid, or WEIDEL'S reaction. With hydrochloric acid and potassium chlorate it gives a white residue which turns violet with ammonia. It does not form any insoluble sodium compound. The silver compound is difficultly soluble in nitric acid.

Epiguanine, $C_6H_7N_5O$, 7-methylguanine,  was first prepared from the urine by KRÜGER.[3] It is crystalline and difficultly soluble in hot water or ammonia. It crystallizes from a hot 33-per cent caustic-soda solution on cooling in broad shining crystals and dissolves readily in hydrochloric or sulphuric acid. It gives a characteristic chloroplatinate crystallizing in six-sided prisms. It is precipitated neither by basic lead acetate nor by basic lead acetate and ammonia. Silver nitrate and ammonia give a gelatinous precipitate. It responds to the xanthine test with nitric acid and alkali. It acts like episarkine with WEIDEL'S test according to FISCHER.

In preparing alloxuric bases from the urine, the fluid is supersaturated with ammonia and precipitated by a silver-nitrate solution. The precipitate is then decomposed with sulphuretted hydrogen. The boiling-hot filtrate is evaporated to dryness and the dried residue treated with 3-per cent sulphuric acid. The purine bases are dissolved, while the uric acid remains undissolved. This filtrate is saturated with ammonia and precipitated by silver-nitrate solution. If instead of precipitating with silver solution we desire to precipitate, according to KRÜGER and WULFF, with copper suboxide, the urine may be heated to boiling, and immediately are added, successively, 100 cc. of a 50-per cent sodium-bisulphite solution and 100 cc. of a 12-per cent copper-sulphate solution for every liter of urine. The thoroughly washed precipitate is decomposed with hydrochloric acid and sulphuretted hydrogen. The uric acid remains in great part on the filter. Further details in regard to the treatment of the solution of the hydrochloric-acid compounds may be found in KRÜGER and SALOMON.[4]

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[1] Thudichum, "Grundzüge d. anal. med. klin. Chemie" (Berlin, 1886); Salomon, Arch. f. (Anat. u.) Physiol., 1882, and Ber. d. deutsch. chem. Gesellsch., **16** and **18**.

[2] Balke, "Zur Kenntniss der Xanthinkörper" (Inaug.-Diss., Leipzig, 1893); Salomon, Zeitschr. f. physiol. Chem., **18**.

[3] Arch. f. (Anat. u.) Physiol., 1894; Krüger and Salomon, Zeitschr. f. physiol. Chem., **24** and **26**.

[4] Zeitschr. f. physiol. Chem., **26**, and also Hoppe-Seyler-Thierfelder's Handbuch, 8. Aufl., 188.

Quantitative Estimation of Purine Bases according to SALKOWSKI.[1] 400–600 cc. of the urine free from protein are first precipitated by magnesia mixture, and then by a 3-per cent silver-nitrate solution as described on page 710. The thoroughly washed silver precipitate is decomposed by sulphuretted hydrogen after being suspended in 600-800 cc. of water with the addition of a few drops of hydrochloric acid. It is heated to boiling and filtered hot, and finally evaporated to dryness on the water-bath. The residue is extracted with 20–30 cc. of hot 3-per cent sulphuric acid and allowed to stand twenty-four hours; the uric acid is filtered off, washed, the filtrate made ammoniacal, and the purine bodies again precipitated by silver nitrate, the precipitate collected on a small chlorine-free filter, washed thoroughly, dried, carefully incinerated, the ash dissolved in nitric acid, and titrated with ammonium sulphocyanide according to VOLHARD'S method. The ammonium-sulphocyanide solution should contain 1.2–1.4 grams per liter, and its strength should be determined by a silver-nitrate solution: 1 part silver corresponds to 0.277 gram nitrogen of purine bases, or to 0.7381 gram purine bases. By this method the uric-acid and purine bases can be simultaneously determined in the same portion of urine.[2]

MALFATTI[3] determines the nitrogen of the purine bases in the hydrochloric-acid filtrate from the separated uric acid. This filtrate is evaporated with magnesia until all the ammonia has been expelled and the residue used for the KJELDAHL determination.

The nitrogen of the purine bases is also determined as the difference between the uric-acid nitrogen and the total nitrogen of the purine bodies of the silver precipitate (CAMERER, ARNSTEIN[4]). Certain objections have been raised against this method but they can be overcome by using the modified method as suggested by KENNAWAY.[5]

According to the method of KRÜGER and SCHMID[6] the uric acid and the purine bases are precipitated as a cuprous compound by copper-sulphate solution and sodium bisulphite. The precipitate is decomposed in sufficient water by sodium sulphide, and the uric acid precipitated from the concentrated filtrate with hydrochloric acid, and the purine bases again precipitated from this filtrate as cuprous or silver compounds. Finally, the nitrogen in the uric-acid part and the part containing the mixture of purine bases is estimated.

Oxaluric Acid, $C_3H_4N_2O_4 = (CON_2H_3).CO.COOH$. This acid, whose relation to uric acid and urea has been spoken of above, does not always occur in the urine, and then only in traces as the ammonium salt. This salt is not directly precipitated by $CaCl_2$ and NH_3, but on boiling it is decomposed into urea and oxalate. In preparing oxaluric acid from urine the latter is filtered through animal charcoal. The oxalurate retained by the charcoal may be obtained by boiling with alcohol.

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[1] Pflüger's Arch., **69**.

[2] In regard to the details we refer the reader to the original paper.

[3] Centralbl. f. innere Med., 1897.

[4] Camerer, Zeitschr. f. Biologie, **26** and **28**; Arnstein, Zeitschr. f. physiol. Chem., **28**.

[5] Journ. of Physiol., **39**.

[6] Zeitschr. f. physiol. Chem., **45**, and Hoppe-Seyler-Thierfelder's Handbuch, 8. Aufl., 590.

Oxalic Acid, $C_2H_2O_4$, or $\begin{matrix}COOH\\ \vdots\\ COOH\end{matrix}$, occurs under physiological conditions in very small amounts in the urine, about 0.02 gram in twenty-four hours (FÜRBRINGER [1]). According to the generally accepted view it exists in the urine as calcium oxalate, which is kept in solution by the acid phosphates present. Calcium oxalate is a frequent constituent of uninary sediments, and also occurs in certain urinary calculi.

The origin of the oxalic acid in the urine is not well known. Oxalic acid when administered is eliminated unchanged, at least in part, by the urine;[2] and as many vegetables and fruits, such as cabbage, spinach, asparagus, sorrel, apples, grapes, etc., contain oxalic acid, it is possible that a part of the oxalic acid of the urine originates directly from the food. That oxalic acid may be formed in the animal body as a metabolic product from proteins or fats follows from the observations of MILLS and LÜTHJE and others, who found that in dogs on an exclusively meat and fat diet, as also in starvation, oxalic acid was eliminated by the urine. The oxalic acid which is eliminated in increased quantity with a diminished oxygen supply and an increased protein catabolism, as found by REALE and BOERI, and also by TERRAY, is supposed to be derived partly from the greater destruction of proteins. Pure protein does not, according to SALKOWSKI and WEGRZYNOWSKI [3] increase the quantity of oxalic acid eliminated; on the contrary, after meat feeding the amount of this acid is increased, due in part to the meat containing oxalic acid (SALKOWSKI). Gelatin and gelatin-yielding tissues seem to increase the excretion of oxalic acid, and the same is also true for fats or at least glycerin (WEGRZYNOWSKI). After feeding nucleins no constant increase in the elimination of oxalic acid has been observed. The statements as to the action of carbohydrates are contradictory. The production of oxalic acid due to an incomplete combustion of the carbohydrates has also been suggested, and the work of HILDEBRANDT and P. MAYER seems to indicate this under abnormal conditions. According to DAKIN,[4] in rabbits an increased elimination of oxalic acid occurs after the introduction of glycollic or glyoxylic acids, and the oxalic acid seems in many cases to be an intermediary product of metabolism, which is further burnt. We cannot exclude the possibility of the formation of oxalic acid in the oxidation of uric acid in the animal body, yet we have no positive proof

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[1] Deutsch. Arch. f. klin. Med., 18. See also Dunlop, Journ. Path. and Bacteriol., 3.

[2] In regard to the behavior of oxalic acid in the animal body, see page 773.

[3] Reale and Boeri, Wien. med. Wochenschr., 1895; Terray, Pflüger's Arch., 65; Salkowski, Berl. klin. Wochenschr., 1900; Wegrzynowski. Zeitschr. f. physiol. Chem., 38 which contains the literature.

[4] Journ. of biol. Chem., 3, 57.

of such a formation.[1] An endogenous as well as an exogenous origin of oxalic acid has also been suggested.

Oxalic acid is best detected and quantitatively determined according to the method suggested by SALKOWSKI: Shaking out the oxalic acid from the acidified urine by means of ether. Detailed account of this can be found in WEGRZYNOWSKI.[2]

Allantoin (GLYOXYLDIUREIDE), $C_4H_6N_4O_3$, $OC<^{NH.CH.HN.CO.NH_2}_{NH.CO}$,

occurs, it is claimed by earlier writers, in the urine of children within the first eight days after birth, and in very small amounts also in the urine of adults (GUSSEROW, ZIEGLER and HERMANN). It is found in rather abundant quantities in the urine of pregnant women (GUSSEROW). According to WIECHOWSKI the urine of adults, if it contains any allantoin at all, has only traces, and he could not detect any in the urine of nurslings or in the amniotic fluid, which does not agree with previous reports. Allantoin has also been found in the urine of suckling calves (WÖHLER), in urine of oxen (SALKOWSKI), and sometimes in the urine of other animals (MEISSNER). WIECHOWSKI has found it in relatively large quantities in the urine of the dog, cat, rabbit and monkey, and he considers that allantoin is a terminal metabolic product in these animals. It is also found, as first shown by VAUQUELIN and LASSAIGNE,[3] in the allantoic fluid of the cow (hence the name). That allantoin is formed from the uric acid in mammalia is almost certain, and the investigations on which this is based have already been given in discussing the decomposition of uric acid.[4] The allantoin thus originates from the purine bodies, and consequently in dogs and other animals the excretion of allantoin is considerably increased, according to MINKOWSKI, COHN, SALKOWSKI, and MENDEL and BROWN,[5] after feeding thymus or pancreas. A strong allantoin excretion is also found in dogs after poisoning with hydrazine (BORISSOW), hydroxylamine, semicarbazide, and aminoguanidine (POHL), and this increase in the excretion of allantoin is

[1] See Wiener, Ergebn. d. Physiol., 1; Tomaszewski, Zeitschr. f. exp. Path. u. Ther., 7; Pohl, *ibid.*, 8; Jastrowitz, Bioch. Zeitschr., 28.

[2] Zeitschr. f. physiol. Chem., 83.

[3] Ziegler and Hermann, see Gusserow, Arch. f. Gynäkol., 3—both cited from Huppert-Neibauer, Harn-Analyse, 10. Aufl., 377; Wöhler, Annal. d. Chem. u. Pharm., 70; Salkowski, Zeitschr. f. physiol. Chem., 42; Meissner, Zeitschr. f. rat. Med. (3), 31; Lassaigne, Annal. de Chim. et Phys., 17; Wiechowski, Hofmeister's Beiträge, 11, and Arch. f. exp. Path. u. Pharm., 60, and Bioch. Zeitschr., 19 and 25.

[4] See footnote 2, page 706.

[5] Minkowski, Arch. f. exp. Path. u. Pharm., 41, and Centralbl. f. innere Med., 1898; Cohn, Zeitschr. f. physiol. Chem., 25; Salkowski, Centralbl. f. d. med. Wissensch., 1898; Mendel and Brown, Amer. Journ. of Physiol., 3.

connected with the nuclein metabolism. POHL[1] has found, in dogs on poisoning with hydrazine, that the liver contained allantoin and that other organs contained traces, while it does not exist in the organs of normal dogs, and he has also detected the formation of allantoin in the autolysis of the intestinal mucosa, liver, thymus, spleen and pancreas. It is very probable that in these cases we are dealing with a destruction of cells and an enzymotic uric acid formation with a subsequent uricolysis with the formation of allantoin. Certain food-stuffs such as milk, wheat bread, peas and beans contain, according to ACKROYD, small amounts of allantoin, which are introduced into the body. Nothing is known about how these traces of allantoin behave in the body. According to PODUSCHKA and MINKOWSKI,[2] allantoin introduced into dogs appears almost entirely in the urine, while in man only a small portion of the ingested substance is eliminated in the urine and seems in the last case to be chiefly burned.

Allantoin is a colorless substance often crystallizing in prisms, difficultly soluble in cold water, easily soluble in boiling water, and also in warm alcohol, but not soluble in cold alcohol or ether. A watery allatoin solution gives no precipitate with silver nitrate alone, but by the careful addition of ammonia a white flocculent precipitate is formed, $C_4H_5AgN_4O_3$, which is soluble in an excess of ammonia and which consists after a certain time of very small, transparent microscopic globules. The dry precipitate contains 40.75 per cent silver. A watery allantoin solution is precipitated by mercuric nitrate. On continued boiling allantoin reduces FEHLING'S solution. It gives SCHIFF'S furfurol reaction less rapidly and less intensely than urea. Allantoin does not give the murexide test.

Allantoin is most easily prepared by the oxidation of uric acid with lead peroxide or potassium permanganate. In preparing allantoin from urine we must proceed differently according to whether we are using the urine of animals comparatively rich in allantoin or whether we are using human urine, which is very poor in allantoin. The same applies to the quantitative estimation of allantoin. As the methods in both cases are complicated and require certain percautions we cannot here enter into a detailed description of them, and we refer to the works of LOEWI and WIECHOWSKI[3] and to the complete handbooks for details. The pre-

[1] Borissow, Zeitschr. f. physiol. Chem., **19**; Pohl. Arch. f. exp. Path. u. Pharm., **46**; Poduschka, *ibid.*, **44**. According to Underhill and Kleiner, Journ. of biol. Chem., **4**, hydrazine has no other action on the excretion of allantoin than that caused by the refusal to take food brought about by the poison.

[2] Ackroyd, Bioch. Journ., **5**; Poduschka, Arch. f. exp. Path. u. Pharm., **44**; Minkowski, *ibid.*, **41**.

[3] Loewi, *ibid.*, **44**; Wiechowski, Hofmeister's Beiträge, **11**, and Arch, f. exp. Path. u. Pharm., **60**; and Bioch. Zeitschr., **19** and **25**.

cipitation of allantoin from the urine can be accomplished by mercuric nitrate and by mercuric acetate solutions, in the presence of sodium acetate.

Glyoxylic Acid, $C_2H_4O_4$, $\begin{matrix}CH(OH)_2\\COOH\end{matrix}$, is produced on boiling allantoin as well as uric acid with alkalies, and also on the oxidation of many substances, among which we can mention creatine and creatinine. It is also of interest that allantoin can be prepared synthetically from glyoxylic acid and urea and that glyoxylic acid yields oxalic acid when introduced into the body. The reports in regard to its occurrence in the urine conflict,[1] as it is readily destroyed in the body, and its passage into the urine is very improbable, or at least only seldom occurs.

Hippuric Acid (BENZOYL-AMINO ACETIC ACID),

$$C_9H_9NO_3 = (C_6H_5CO)HN \cdot CH_2COOH.$$

This acid decomposes into benzoic acid and glycocoll on boiling with mineral acids or alkalies, and also in the putrefaction of the urine. The reverse of this occurs if these two components are heated in a sealed tube, according to the following equation: $C_6H_5COOH + NH_2.CH_2.COOH = C_6H_5.CO.NH.CH_2.COOH + H_2O$. This acid may be synthetically prepared from benzamide and monochloracetic acid, $C_6H_5.CO.NH_2 + CH_2Cl.COOH = C_6H_5.CO.NH.CH_2.COOH + HCl$, and in various other ways, but most simply from glycocoll and benzoyl chloride in the presence of alkali.

Hippuric acid occurs in large amounts in the urine of herbivora, but only in small quantities in that of carnivora. The quantity of hippuric acid eliminated in human urine on a mixed diet is usually less than 1 gram per day; as an average it is 0.7 gram. After eating freely of vegetables and fruit, especially such fruit as plums, the quantity may be more than 2 grams. Hippuric acid is also found in the perspiration, the blood, the suprarenal capsule of oxen, and in ichthyosis scales. Nothing is positively known in regard to the quantity of hippuric acid in the urine in disease.

The Formation of Hippuric Acid in the Organism. Benzoic acid and also the substituted benzoic acids are converted into hippuric acid and substituted hippuric acids within the body. Moreover, those bodies are transformed into hippuric acid which by oxidation (toluene, cinnamic acid, hydrocinnamic acid) or by reduction (quinic acid) are converted into benzoic acid. The question of the origin of hippuric acid is therefore connected with the question of the origin of benzoic acid; the formation of the second component, glycocoll, from the protein substances in the body is unquestionable.

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[1] The literature on the occurrence and detection of glyoxylic acid in the urine can be found in Granström, Hofmeister's Beiträge, 11.

Hippuric acid is found in the urine of starving dogs (SALKOWSKI), also in dog's urine after a diet consisting entirely of meat (MEISSNER and SHEPARD, SALKOWSKI, and others [1]). It is evident that the benzoic acid originates in these cases from the proteins, and it is generally admitted that it is produced by the putrefaction of proteins in the intestine. Among the products of the putrefaction of protein outside of the body SALKOWSKI found phenylpropionic acid, $C_6H_5.CH_2.CH_2.COOH$, which is oxidized in the organism to benzoic acid and eliminated as hippuric acid after combining with glycocoll. Phenylpropionic acid seems to be formed from the phenylalanine. The supposition that the phenylpropionic acid is produced from tyrosine by putrefaction of the intestine has not been substantiated by the researches of BAUMANN, SCHOTTEN, and BAAS.[2] The importance of putrefaction in the intestine in producing hippuric acid is evident from the fact that after thoroughly disinfecting the intestine of dogs with calomel the hippuric acid disappears from the urine (BAUMANN[3]).

The large quantity of hippuric acid present in the urine of herbivora is partly explained by the specially active processes of putrefaction going on in the intestine of these animals. According to VASILIU [4] this can hardly be correct, because, as he has found, by feeding sheep with casein, this would require a too intense putrefaction of the protein (indeed 40 per cent of it). This author's explanation lies in part that in the herbivora only a small part of the phenylalanine is burnt, and is used to a greater extent in the formation of hippuric acid than in man and carnivora, and in part by the fact that the food of herbivora contains larger quantities of a non-nitrogenous mother-substance of the benzoic acid. There is hardly any doubt that the hippuric acid in human urine after a mixed diet, and especially after a diet of vegetables and fruits, originates in part from the aromatic substances, e. g., quinic acid.

The view proposed by WEISS and others that a parallelism exists between the excretion of hippuric acid and uric acid in that an increase in the first is followed by a diminution in the second, and that, for example, quinic acid produces a diminution in the excretion of uric acid corresponding to the increased formation of hippuric acid (WEISS, LEWIN), cannot be considered as sufficiently proven (HUPFER).[5]

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[1] Salkowski, Ber. d. deutsch. chem. Gesellsch., 11; Meissner and Shepard, Untersuch. über das Entstehen der Hippursäure im thierschen Organismus. Hanover. 1886.

[2] E. and H. Salkowski, Ber. d. deutsch. Chem. Gesellsch., 12; Baumann, Zeitschr. f. physiol. Chem., 7; Schotten, *ibid.*, 8; Baas, *ibid.*, 11.

[3] *Ibid.*, 10, 131.

[4] Vasiliu, Mitt. d. landwirt. Inst. Breslau, Bd. 4, 1907.

[5] Weiss, Zeitschr. f. physiol. Chem., 25, 27, 28; Lewin, Zeitschr. f. klin. Med., 42;

As the thorough investigations of WIECHOWSKI teach, the synthesis of hippuric acid does not stand in any direct relation to the extent of protein metabolism; it varies, on the contrary, with the duration of circulation of benzoic acid and the quantity of glycocoll present in the body. The amount of the latter in intermediary metabolism is so great that in rabbits, on the administration of benzoic acid, more than one-half of the total urine nitrogen may exist as glycocoll. MAGNUS-LEVY [1] found in rabbits and sheep up to 27.8 per cent of the total nitrogen as hippuric-acid nitrogen, and both investigators have found so much hippuric-acid nitrogen that it could not be accounted for by the glycocoll preformed from the proteins, which amounts to about 4–5 per cent of the total nitrogen of the protein of the food and body.

In carnivora (dog) and man the conditions are different, according to BRUGSCH and R. HIRSCH, FEIGIN and BRUGSCH, as in these cases there is no more glycocoll available for hippuric acid formation than is split off from the proteins on hydrolysis. According to the investigations of LEWINSKI [2] this does not seem to be correct, at least not for man. After abundant introduction of benzoic acid in man about 34 per cent of the total nitrogen may be excreted as hippuric acid and in a recent investigation he was able to obtain 50.5 grams pure crystalline hippuric acid from the 24-hour urine of a man after feeding sodium benzoate.

The abundant production of hippuric acid in herbivora induced ABDERHALDEN, GIGON and STRAUSS to investigate the comparative supply of certain amino-acids in carnivora and herbivora, and they found in cats, rabbits and hens that the percentage quantity of glycocoll split off from the entire organism (with the exception of the intestinal contents and fat and feathers) by hydrolysis was the same, namely 2.33 to 3.34 per cent of the proteins. In order to account for the large quantity of glycocoll which can be eliminated as hippuric acid, we must admit of a formation of glycocoll. That this occurs in animals fed with benzoic acid has been recently proved by ABDERHALDEN and HIRSCH by very conclusive experiments. It can be assumed that the benzoic acid combines with higher amino-acids and that the hippuric acid is formed from this combination. The investigations of MAGNUS-LEVY to prove this assumption, where he used benzoylated higher amino-acids, have not

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Hupfer, Zeitschr. f. physiol. Chem., **37**. See also Wiener, "Die Harnsäure," Ergebnisse der Physiol., **1**, Abt. 1.

[1] Wiechowski, Hofmeister's Beiträge, **7** (literature); A. Mangus-Levy, Münch. med. Wochenschr., 1905; Ringer, Journ. of biol. Chem., **10**; Epstein and Bookman, *ibid.*, **10**.

[2] Brugsch and Hirsch, Zeitschr. f. exp. Path. u. Therap., **3**; Brugsch, Maly's Jahresber., **37**, 621, and Bioch. Centralbl., **8**, 336; Feigin, Maly's Jahresber., **36**, 631; Lewinski, Arch. f. exp. Path. u. Pharm., **58** and **61**.

given support to this assumption; EPSTEIN and BOOKMAN [1] found nevertheless in experiments with rabbits after feeding with benzoyl-leucine that a great elimination of hippuric acid occurred which they consider as a formation of glycocoll from this leucine. Free leucine on the contrary does not increase the hippuric acid elimination.

The kidneys may be considered in dogs as special organs for the synthesis of hippuric acid (SCHMIEDEBERG and BUNGE [2]). In other animals as in rabbits, the formation of hippuric acid seems to take place in other organs, such as the liver and muscles. The synthesis of hippuric acid is therefore not exclusively limited to any special organ, though perhaps in some species of animals it may be more abundant in one organ than in another.

Properties and Reactions of Hippuric Acid. This acid crystallizes in semi-transparent, long, four-sided, milk-white, rhombic prisms or columns, or in needles by rapid crystallization. They dissolve in 600 parts cold water, but more easily in hot water. They are easily soluble in alcohol, but with difficulty in ether. The acid dissolves more easily (about 12 times) in acetic ether than in ethyl ether. Petroleum-ether does not dissolve hippuric acid.

On heating hippuric acid it first melts at 187.5° C. to an oily liquid which crystallizes on cooling. On continued heating it decomposes, producing a red mass and a sublimate of benzoic acid, with the generation, first, of a peculiar pleasant odor of hay and then an odor of hydrocyanic acid. Hippuric acid is easily differentiated from benzoic acid by this behavior, also by its crystalline form and its insolubility in petroleum ether. Hippuric acid and benzoic acid both give LÜCKE's reaction, namely, they generate an intense odor of nitrobenzene when evaporated to dryness with nitric acid and when the residue is heated with sand in a glass tube. Hippuric acid in most cases forms crystallizable salts, with bases. The combinations with alkalies and alkaline earths are soluble in water and alcohol. The silver, copper, and lead salts are soluble with difficulty in water; the ferric salt is insoluble.

Hippuric acid is best prepared from the fresh urine of a horse or cow. The urine is boiled a few minutes with an excess of milk of lime. The liquid is filtered while hot, concentrated and then cooled, and the hippuric acid precipitated by the addition of an excess of hydrochloric acid. The crystals are pressed, dissolved in milk of lime by boiling, and treated as above; the hippuric acid is precipitated again from the concentrated

[1] Abderhalden, Gigon and Strauss, Zeitschr. f. physiol. Chem., **51**; Abderhalden and Hirsch, *ibid.*, **78**; Mangus-Levy, Bioch. Zeitschr., **6**; Epstein and Bookman, Journ. of biol. Chem., **13**.

[2] Arch. f. exp. Path. u. Pharm., **6**; also A. Hoffmann, *ibid.*, **7**, and Kochs, Pflüger's Arch., **20**; Bashford and Cramer, Zeitschr. f. physiol. Chem., **35**.

filtrate by hydrochloric acid. The crystals are purified by recrystallization and decolorized, when necessary, by animal charcoal.

The quantitative estimation of hippuric acid in the urine may be performed by the following method (BUNGE and SCHMIEDEBERG): The urine is first made faintly alkaline with soda, evaporated nearly to dryness, and the residue thoroughly extracted with strong alcohol. After the evaporation of the alcohol the residue is dissolved in water, the solution acidified with sulphuric acid, and completely extracted by agitating (at least five times) with fresh portions of acetic ether. The acetic ether is then repeatedly washed with water, which is removed by means of a separatory funnel, then evaporated at a medium temperature and the dry residue treated repeatedly with petroleum-ether, which dissolves the benzoic acid, oxyacids, fats, and phenols, while the hippuric acid remains undissolved. This residue is now dissolved in a little warm water and evaporated at 50–60° C. to crystallization. The crystals are collected on a small weighed filter. According to HENRIQUES and SÖRENSEN the acidified urine can be directly shaken out with acetic ether, the residue after evaporation of the acetic ether boiled with hydrochloric acid in order to split the hippuric acid into benzoic acid and glycocoll and the quantity of nitrogen in the latter determined by a formol titration. Other methods have recently been suggested by FOLIN and FLANDERS, by STEENBOCK and by HRYNTSCHAK.[1]

Phenaceturic Acid, $C_{10}H_{11}NO_3 = C_6H_5.CH_2.CO.NH.CH_2.COOH$. This acid, which is produced in the animal body by a combination of glycocoll with the phenylacetic acid, $C_6H_5.CH_2.COOH$, formed in the putrefaction of the proteins, has been prepared from horse's urine by SALKOWSKI,[2] but it probably also occurs in human urine. According to VASILIU [3] it is just as important a constituent of the urine of herbivora as hippuric acid is.

Benzoic Acid, $C_7H_6O_2$ or $C_6H_5.COOH$, is found in rabbit's urine and sometimes, though in small amounts, in dog's urine (WEYL and v. ANREP). According to JAARSVELD and STOKVIS and to KRONECKER it is also found in human urine in diseases of the kidneys. The occurrence of benzoic acid in the urine seems to be due to a fermentative decomposition of hippuric acid. Such a decomposition may very easily occur in an alkaline urine or in one containing proteid (VAN DE VELDE and STOKVIS). In certain animals—pigs and dogs—the kidneys, according to SCHMIEDEBERG and MINKOWSKI,[4] contain a special enzyme, SCHMIEDEBERG's *histozym*, which splits the hippuric acid with the separation of benzoic acid.

Ethereal Sulphuric Acids. In the putrefaction of proteins in the intestine, phenols—whose mother-substance is considered to be tyrosine—and also indol and skatol are produced. These phenols directly, and the two last-named bodies after they have been oxidized respectively into

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[1] Bunge and Schmiedeberg, Arch. f. exp. Path. u. Pharm., **6**; Henriques and Sörensen, Zeitschr. f. physiol. Chem., **64**; Folin and Flanders, Journ. of biol. Chem., **11**; Steenbock, *ibid.*, **11**; Hryntschak, Bioch. Zeitschr., **43**.

[2] Zeitschr. f. physiol. Chem., **9**.

[3] Mitteil. d. landw. Inst., Breslau, **4**.

[4] Weyl and v. Anrep, Zeitschr. f. physiol. Chem., **4**; Jaarsveld and Stokvis, Arch. f. exp. Path. u. Pharm., **10**; Kronecker, *ibid.*, **16**; Van de Velde and Stokvis, *ibid.*, **17**; Schmiedeberg, *ibid.*, **14**, 379; Minkowski, *ibid.*, **17**.

indoxyl and skatoxyl, pass into the urine as ethereal sulphuric acids after uniting with sulphuric acid. The most important of these ethereal acids are *phenol-* and *cresol-sulphuric acids*—which were formerly also called phenol-forming substances—*indoxyl-* and *skatoxyl-sulphuric acids.* To this group also belong *pyrocatechin-sulphuric acid,* which occurs only in very small amounts in human urine, and *hydroquinone-sulphuric acid,* which appears in the urine after poisoning with phenol, and under physiological conditions perhaps other ethereal acids occur which have not been isolated. The ethereal sulphuric acids of the urine were discovered and specially studied by BAUMANN.[1] The quantity of these acids in human urine is small, while horse's urine contains larger quantities. According to the determinations of v. D. VELDEN the quantity of ethereal sulphuric acid in human urine in twenty-four hours varies between 0.094 and 0.620 gram. C. TOLLENS found an average of 0.18 gram. The relation of the sulphate-sulphuric acid *A* to the conjugated sulphuric acid *B*, in health, is on an average 10:1. It undergoes such great variations, as found by BAUMANN and HERTER,[2] and after them by many other investigators, that it is hardly possible to consider the average figures as normal. After taking phenol and certain other aromatic substances, as well as when putrefaction within the organism is general, the elimination of ethereal sulphuric acid is greatly increased. On the contrary, it is diminished when the putrefaction in the intestine is reduced or prevented. For this reason it may be greatly diminished by carbohydrates and exclusive milk diet.[3] The intestinal putrefaction and the elimination of ethereal sulphuric acid have also been diminished in some cases by certain therapeutic agents which have an antiseptic action; still the investigators do not agree in their reports.[4]

Great importance has been given to the relation between the total sulphuric acid and the conjugated sulphuric acid, or between the conjugated sulphuric acid and the sulphate-sulphuric acid, in the study of the intensity of the putrefaction in the intestine under different conditions. Several investigators, F. MÜLLER, SALKOWSKI, and v. NOORDEN.[5]

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[1] Pflüger's Arch., **12** and **18**.

[2] v. d. Velden, Virchow's Arch., **70**; Tollens, Zeitschr. f. physiol. Chem., **67**; Herter, Zeitschr. f. physiol. Chem., **1**.

[3] See Hirschler, Zeitschr. f. physiol. Chem., **10**; Biernacki, Deutsch. Arch. f. klin. Med., **49**; Rovighi, Zeitschr. f. physiol. Chem., **16**; Winternitz, *ibid.*, and Schmitz, *ibid.*, **17** and **19**.

[4] See Baumann and Morax, Zeitschr. f. physiol. Chem., **10**; Steiff, Zeitschr. f. klin. Med., **16**; Rovighi, l. c.; Stern, Zeitschr. f. Hyg., **12**; and Bartoschewitsch, Zeitschr. f. physiol. Chem., **17**; Mosse, *ibid.*, **23**.

[5] Müller, Zeitschr. f. klin. Med., **12**; v. Noorden, *ibid.*, **17**; Salkowski, Zeitschr. f. physiol. Chem., **12**.

consider correctly that this relation is only of secondary value, and that it is more correct to consider the absolute value. It must be remarked that the absolute values for the conjugated sulphuric acid also undergo great variation, so that it is at present impossible to give the upper or lower limit for the normal value.

Phenol- and p-Cresol-sulphuric Acids, $C_6H_5.O.SO_2.OH$ and $C_6H_4\begin{cases}O.SO_2.OH\\CH_3\end{cases}$ These acids are found as alkali salts in human urine, in which also orthocresol has been detected. The quantity of cresol-sulphuric acid is considerably greater than of phenol-sulphuric acid. In the quantitative estimation the phenols are set free from the two ethereal acids and determined together as tribromphenol. The quantity of phenols which are separated from the ethereal-sulphuric acids of the urine amounts to 17–51 milligrams in the twenty-four hours (MUNK). In nine case investigated by SIEGFRIED and ZIMMERMANN[1] they found in the urine of healthy students in 1500 cc. urine an average of 44.6 milligrams phenols, of which 26 milligrams was cresol and 18.6 milligrams was phenol. After the ingestion of carbolic acid, which is in great part converted by synthesis within the organism into phenol-sulphuric acid, also into pyrocatechin- and hydroquinon-sulphuric acid[2] or when the amount of sulphuric acid is not sufficient to combine with the phenol, it forms phenol-glucuronic acid,[3] the quantity of phenols and ethereal-sulphuric acids in the urine is considerably increased at the expense of the sulphate-sulphuric acid. The same is also true of other phenols. The cresol is in great part changed into phenol in dogs, according to SIEGFRIED and ZIMMERMANN.[4]

An increased elimination of phenol-sulphuric acids occurs in active putrefaction in the intestine with stoppage of the contents of the intestine, as in ileus, diffused peritonitis with atony of the intestine, or tuberculous enteritis, but not in simple obstruction. The elimination is also increased by the absorption of the products of putrefaction from purulent wounds or abscesses. An increased elimination of phenol has been observed in a few other cases of diseased conditions of the body.[5]

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[1] Munk, Pflüger's Arch., **12**; Siegfried and Zimmermann, Bioch. Zeitschr., **34**.

[2] See Baumann, Pflüger's Arch., **12** and **13**, and Baumann and Preusse, Zeitschr. f. physiol. Chem., **3**, 156.

[3] Schmiedeberg, Arch. f. exp. Path. u. Pharm., **14**; C. Tollens, Zeitschr. f. physiol. Chem., **67**.

[4] Bioch. Zeitschr., **46**.

[5] See G. Hoppe-Seyler, Zeitschr. f. physiol. Chem., **12** (this contains also all references to the literature on this subject); Fedeli, Moleschott's Untersuch., **15**.

The alkali salts of phenol- and cresol-sulphuric acids crystallize in white plates, similar to mother-of-pearl, which are rather freely soluble in water. They are soluble in boiling alcohol, but only slightly soluble in cold alcohol. On boiling with dilute mineral acids they are decomposed into sulphuric acid and the corresponding phenol.

Phenol-sulphuric acids have been synthetically prepared by BAUMANN from potassium pyrosulphate and potassium phenolate or *p*-cresolate. For the method of their preparation from urine, which is rather complicated, and also for the known phenol reactions, the reader is referred to other text-books. The quantitative estimation of the phenols from these etheral sulphuric acids is now ordinarily done by the following methods:

KOSSLER and PENNY'S method with NEUBERG'S [1] modification. The liquid containing phenol is treated with N/10 caustic soda until strongly alkaline, warmed on the water-bath in a flask with a glass stopper, and then treated with an excess of N/10 iodine solution, the quantity being exactly measured. Sodium iodide is first formed and then sodium hypoiodite, which latter forms tri-iodophenol with the phenol according to the following equation:

$$C_6N_5OH+3NaIO=C_6H_2I_3.OH+3NaOH.$$

On cooling, acidify with sulphuric acid and determine the excess of iodine by titration with N/10 sodium thiosulphate solution. This process is also available for the estimation of paracresol. Each cubic centimeter of the iodine solution used is equivalent to 1.5670 milligrams of phenol or 1.8018 milligrams of cresol. As the determination does not give any idea as to the variable proportions of the two phenols, the quantity of iodine used must be calculated as one or the other of the two phenols. Before such a determination is carried out, the concentrated urine is first distilled after acidification with sulphuric acid and the distillate purified by precipitation with lead, and distilled again (NEUBERG). MOOSER has raised objections against the use of sulphuric acid and recommends instead the use of phosphoric acid. In regard to the dispute which has arisen between NEUBERG and MOOSER [2] as well as to the details of NEUBERG'S method we must refer to the original publications and to larger handbooks.

For the separate estimation of phenol and *p*-cresol in the urine a special method has been suggested by SIEGFRIED and ZIMMERMANN.[3] The principle of the method consists in the two following estimations: 1. The quantity of bromine necessary to convert the phenol and cresol into tribromphenol and tribromcresol is determined. 2. The quantity of bromine necessary to convert the phenol into tribromphenol and the

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[1] Kossler and Penny, Zeitschr. f. physiol. Chem., 17; Neuberg, *ibid.*, 27.

[2] Mooser, Zeitschr. f. physiol. Chem., 63, with Liechti, *ibid.*, 73; Neuberg and Hildesheimer, Bioch. Zeitschr., 28; Marie Hensel, Zeitschr. f. physiol. Chem., 78.

[3] Siegfried and Zimmermann, Bioch. Zeitschr., 29, 34 and 38; see also Ditz and Bardach, *ibid.*, 37 and 42.

cresol into dibromcresol under exactly observed conditions is determined and from the quantities of bromine by weight (B_1 and B_2) quantities by weight of phenol and cresol can be calculated. In regard to the procedure as well as to the necessary solutions we refer to the original publication.

The methods for the separate determination of the conjugated sulphuric acid and the sulphate-sulphuric acid will be spoken of later in connection with the determination of the sulphuric acid of the urine.

Pyrocatechin-sulphuric Acid. This acid was first found in horse's urine in rather large quantities by BAUMANN. It occurs in human urine only in the very smallest amounts, and perhaps not constantly, but it is present abundantly in the urine after taking phenol, pyrocatechin, or protocatechuic acid.

With an exclusively meat diet this acid does not occur in the urine, and it therefore must originate from vegetable food. It probably originates from the protocatechuic acid, which, according to PREUSSE, passes in part into the urine as pyrocatechin-sulphuric acid. This acid may also perhaps be formed by the oxidation of phenol within the organism (BAUMANN and PREUSSE [1]).

Pyrocatechin, or *o*-DIOXYBENZENE, $C_6H_4(OH)_2$, was first observed in the urine of a child (EBSTEIN and J. MÜLLER). The reducing body ALCAPTON, first found by BÖDEKER [2] in human urine and which was considered for a long time as identical with pyrocatechin, is in most cases probably *homogentisic acid* (see below).

Pyrocatechin crystallizes in prisms which are soluble in alcohol, ether, and water. It melts at 102–104° C., and sublimes in shining plates. The watery solution becomes green, brown, and finally black in the presence of alkali and the oxygen of the air. If very dilute ferric chloride is treated with tartaric acid and then made alkaline with ammonia, and this added to a watery solution of pyrocatechin, we obtain a violet or cherry-red liquid which becomes green on adding excess of acetic acid. Pyrocatechin is precipitated by lead acetate. It reduces an ammoniacal silver solution at the ordinary temperature, and with heat reduces alkaline copper-oxide solutions but does not reduce bismuth oxide.

A urine containing pyrocatechin, if exposed to the air, especially when alkaline, quickly becomes dark and reduces alkaline copper solutions when heated. In detecting pyrocatechin in the urine it is concentrated when necessary, filtered, boiled with the addition of sulphuric acid to remove the phenols, and repeatedly shaken, after cooling, with ether. The ether is distilled from the several ethereal extracts, the residue neutralized with barium carbonate and shaken again with ether. The pyrocatechin which remains after evaporating the ether may be purified by recrystallization from benzene.

Hydroquinone, or *p*-DIOXYBENZENE, $C_6H_4(OH)_2$, often occurs in the urine after the use of phenol (BAUMANN and PREUSSE). The dark color which certain urines, so-called "carbolic urines," assume in the air is due to decomposition products. Hydroquinone does not occur as a normal constituent of urine, but only after the administration of hydroquinone; and according to LEWIN,[3] it may be found in the urine of rabbits as an ethereal-sulphuric acid, being a decomposition product of arbutin.

Hydroquinone forms rhombic crystals which are readily soluble in water, alcohol, and ether. It melts at 169° C. Like pyrocatechin, it easily reduces metallic oxides. It acts like pyrocatechin with alkalies, but is not precipitated by lead acetate. It is oxidized into quinone by ferric chloride and other oxidiz-

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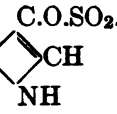
[1] Baumann and Herter, Zeitschr. f. physiol. Chem., **1**; Preusse, *ibid.*, **2**; Baumann, *ibid.*, **3**.

[2] Ebstein and Müller, Virchow's Arch., **62**; Bödeker, Zeitschr. f. rat. Med. (3), **7**.

[3] Lewin, Virchow's Arch., **92**; Bass, Zeitschr. f. exp. Path. u. Ther., **10**.

ing agents, and quinone can be detected by its peculiar odor. Hydroquinone-sulphuric acid is detected in the urine by the same methods as pyrocatechin sulphuric acid.

Indoxyl-sulphuric Acid, $C_8H_7NSO_4$,  , also called URINE INDICAN, formerly called UROXANTHINE (HELLER), occurs as an alkali-salt in the urine. This acid is the mother-substance of a great part of the indigo of the urine. The quantity of indigo which can be separated from the urine is considered as a measure of the quantity of indoxyl-sulphuric acid (and indoxyl-glucuronic acid) contained in the urine. This amount, according to JAFFÉ, for man is 5–20 milligrams per twenty-four hours, and 0.9–37.6 milligrams according to MAILLARD.[1] Horse's urine contains about twenty-five times as much indigo-forming substance as human urine.

Indoxyl-sulphuric acid is derived, as previously mentioned (page 515), from indol, which is first oxidized in the body into indoxyl and is then conjugated with sulphuric acid. After subcutaneous injection of indol the elimination of indican is considerably increased (JAFFÉ, BAUMANN and BRIEGER, and others). It is also increased by the introduction in the animal organism of orthonitrophenolpropiolic acid (G. HOPPE-SEYLER[2]). Indol is formed by the putrefaction of proteins. The putrefaction of secretions rich in protein in the intestine also explains the occurrence of indican in the urine during starvation. Gelatin, on the contrary, does not increase the elimination of indican.

An abnormally increased elimination of indican occurs in those diseases where the small intestines are obstructed, causing an increased putrefaction and thus producing an abundance of indol. Such an increased elimination of indican occurs on tying the small intestine of a dog, but not the large intestine (JAFFÉ), an observation which has been recently confirmed by ELLINGER and PRUTZ.[3] They removed an intestine loop in dogs and replaced it in a reversed position, the distal end of the loop being attached to the proximal end of the intestine, and in this manner. by the inverted peristalsis so obtained, they effected a disturbance in the movement of the intestinal contents. It was shown that this obstruction in the small intestine caused an increased elimination of indican. while an obstruction in the large intestine showed no such action.

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[1] Jaffé, Pflüger's Arch., 3; Maillard, Journ. de Physiol. et de Pathol., 12.

[2] Jaffé, Centralbl. f. d. med. Wissensch., 1872; Baumann and Brieger, Zeitschr. f. physiol. Chem., 3; G. Hoppe-Seyler, *ibid.*, 7 and 8. See also Porcher and Hervieux, Journ. de Phyisol., 7.

[3] Jaffé, Virchow's Arch., 70; Ellinger and Prutz, Zeitschr. f. physiol. Chem., 38.

The putrefaction of proteins in other organs and tissues besides the intestine may also cause an increase in the indican of the urine. Certain investigators, BLUMENTHAL, ROSENFELD, and LEWIN, claim to have shown that an increased excretion of indican can also be brought about without putrefaction by an increased destruction of tissue in starvation and also after phlorhizin poisoning; but these statements are vehemently opposed by other investigators, such as P. MAYER, SCHOLZ, and ELLINGER, and are improbable. The indol, it seems, is not formed from the tryptophane (indolaminopropionic acid) as intermediary step in the demolition of the proteins in the animal body, but rather from the putrefaction of the tryptophane in the intestine. GENTZEN[1] has also shown that tryptophane introduced subcutaneously or per os into the body does not lead to an indicanuria, but only when it is exposed to bacterial decomposition in the large intestine. The reports as to the elimination of indican after oxalic-acid poisoning are conflicting. After poisoning with oxalic acid HARNACK and v. LEYEN found an increased indican elimination, and MORACZEWSKI believes he has proven a certain parallelism between the quantity of indican and the quantity of oxalic acid in diabetes. SCHOLZ,[2] on the contrary, obtained no increase in the excretion of indican after oxalic-acid poisoning.

The excretion of indican is, as above stated, increased by the introduction of indol, but also by indoxyl or indoxyl-carboxylic acid. Indol-carboxylic acid, on the contrary, does not yield indican, but, according to PORCHER and HERVIEUX, another chromogen. BENEDICENTI has also shown that indigo blue or analogous blue or green pigments are produced only from those derivatives of indol which, like *n*-methyl indol $C_6H_4\langle^{CH}_{N.CH_3}\rangle CH$, *α*-naphtindol, $C_{10}H_6\langle^{CH}_{NH}\rangle CH$ or *n*-methylindolin, $C_6H_4\langle^{CH_2}_{N.CH_3}\rangle CH_2$, do not have the hydrogen atoms of the two methine groups substituted by alkyl. From those derivatives in which one or two hydrogen atoms are substituted by alkyl, such as skatol, *α*-methyl indol, dimethyl indol, $C_6H_4\langle^{C.CH_3}_{NH}\rangle C.CH_3$, and bz. 3, p. 2-dimethyl indol, $CH_3.C_6H_3\langle^{CH}_{NH}\rangle C.CH_3$, red pig-

[1] Blumenthal, Arch. f. (Anat. u.) Physiol., 1901, Suppl., and 1902, with Rosenfeld, Charité annalen, **27**, and Hofmeister's Beiträge, **5**; Lewin, Hofmeister's Beiträge, **1**; Mayer, Arch. f. (Anat. u.) Physiol., 1902, Zeitschr. f. klin. Med., **47**, and Zeitschr. f. physiol. Chem., **29**, **32**; Scholz, *ibid.*, **38**; Ellinger, *ibid.*, **39**; Gentzen, "Ueber die Vorstufen des Indols bei der Eiweissfäulnis im Thierkörper," Inaug.-Dissert., Königsberg, 1904.

[2] Harnack, Zeitschr. f. physiol. Chemie, **29**; Scholz, l. c., Moraczewski, Centralbl. f. innere Med., 1903.

ments are produced, a behavior which PORCHER and HERVIEUX [1] have observed in several alkyl-substituted indols.

An increased elimination of indican has been observed in many diseases,[2] and in these cases the quantity of phenol eliminated is also generally increased. A urine rich in phenol is not always rich in indican.

The potassium salt of indoxyl-sulphuric acid, which was prepared pure by BAUMANN and BRIEGER from the urine of dog fed on indol, has subsequently been prepared synthetically by BAUMANN and THESEN,[3] by fusing phenyl-glycine-orthocarboxylic acid with alkali and then from this producing the indoxylsulphate by means of potassium pyrosulphate. It crystallizes in colorless, shining plates or leaves which are easily soluble in water, but less readily in alcohol. It is split by mineral acids into sulphuric acid and indoxyl. The latter without access of air passes into a red compound, indoxyl red, but in the presence of oxidizing reagents is converted into indigo blue: $2C_8H_7NO+2O=C_{16}H_{10}N_2O_2+2H_2O$. The detection of indican is based on this last fact.

For the rather complicated preparation of indoxyl-sulphuric acid as potassium salt from urine the reader is referred to other text-books. For the detection of indican in urine in ordinary cases the following method of JAFFÉ-OBERMAYER, which also serves as an approximate test for the quantity of indican, is sufficient.

JAFFÉ-OBERMAYER'S *Indican Test.* JAFFÉ uses chloride of lime as the oxidizing agent, while OBERMAYER employs ferric chloride. Other oxidizing agents have been suggested, such as potassium permanganate, potassium dichromate, alkali chlorate, and hydrogen peroxide (the latter suggested by PORCHER and HERVIEUX [4]). With OBERMAYER'S reagent the test is performed as follows:

The acid urine (if alkaline it must be acidified with acetic acid) (ELLINGER) is precipitated with basic lead acetate, 1 cc. for every 10 cc. of the urine. 20 cc. of the filtrate are treated in a test-tube with an equal volume of pure concentrated hydrochloric acid (specific gravity 1.19) which contains 2–4 grams ferric chloride to the liter, and 2–3 cc. chloroform are added and the mixture immediately thoroughly shaken. The chloroform is thereby colored more or less blue, depending upon the amount of indican. Besides indigo blue we may also have indigo red produced, whose formation has been explained in various ways. The

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[1] The work of Porcher and Hervieux can be found in Compt. Rend., **145**, Compt. rend. soc. biol., **62**, and Bull. soc. chim. (4), 1; Benedicenti, Zeitschr. f. physiol. Chem., **53** and Arch. f. exp. Path. u. Pharm., 1908, Suppl. (Schmiedeberg's Festschr.).

[2] See Jaffé, Pflüger's Arch., **3**; Senator, Centralbl. f. d. med. Wissensch., 1877; G. Hoppe-Seyler, Zeitschr. f. physiol. Chem., **12** (contains older literature; also Berl., klin. Wochenschr., 1892.

[3] Baumann with Brieger, Zeitschr. f. physiol. Chem., **3**; with Thesen, *ibid.*, **23**.

[4] Jaffé, Pflüger's Arch., **3**; Obermeyer, Wien. klin. Wochenschr., 1890; Salkowski, Zeitschr. f. physiol. Chem., **57**; Porcher and Hervieux, Zeitschr. f. physiol. Chem., **39**.

quantity of indigo red becomes greater the more slowly the oxidation takes place, and especially when the decomposition takes place in the warmth (see the works of ROSIN, BOUMA, WANG, MAILLARD, ELLINGER and HERVIEUX [1]).

According to ELLINGER the source of the indigo-red formation may be the isatin that is produced by the superoxidation of the indoxyl by the action of the reagent, and this isatin forms indigo red with the indoxyl in the hydrochloric-acid solution. MAILLARD, on the contrary, is of the view that the blue substance which is taken up by the chloroform from the urine mixed with hydrochloric acid is not indigotin (indigo-blue), but another substance, called by him hemi-indigotin, which in alkaline solution polymerizes immediately into indigotin, while in acid reaction it is converted into indirubin (indigo red).

The chloroform solution of indigo obtained in the indican test may be used in the quantitative colorimetric determination by comparison with a solution of indigo in chloroform of known strength (KRAUSS and ADRIAN). WANG and others convert the indigo into indigo-sulphonic acid by concentrated sulphuric acid and titrate with potassium permanganate. There is still doubt as to the surest and most trustworthy method for the determination of indican, and especially as to the question how the indigo residue is to be washed (see WANG, BOUMA, ELLINGER, and SALKOWSKI [2]), and for this reason we shall refer only to the works cited above.

Because of the difficulty arising from the production of indirubin in addition to indigotin, BOUMA has recommended the conversion of all the indoxyl into indirubin by boiling the urine with hydrochloric acid containing isatin. The indirubin can be taken up by chloroform and determined by titration with potassium permanganate and sulphuric acid after purification of the chloroform residue. OERUM [3] has also worked out a colorimetric method of estimation based upon BOUMA'S method.

Indol seems also to pass into the urine as a glucuronic acid, *indoxyl-glucuronic acid* (SCHMIEDEBERG). Such an acid has been found in the urine of animals after the administration of the sodium-salt of *o*-nitrophenylpropiolic acid (G. HOPPE-SEYLER). PORCHER and HERVIEUX [4] have obtained indoxyl sulphuric acid in dogs and asses under similar conditions.

Free indigo, and in fact indirubin as well as indigotin, occur in rare cases in the undecomposed urine. GRÖBER and WANG have recently observed such cases. According to STEENSMA [5] traces of free indol occur always in the urine.

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[1] Rosin, Virchow's Arch., **123**; Bouma, Zeitschr. f. physiol. Chem., **27**, **30**, **32**, **39**; Wang, *ibid.*, **25**, **27**, **28**; Ellinger, *ibid.*, **38** and **41**; Maillard, Bull. soc. chim., Paris (3), **29**, and Compt. Rend., **136**; also L'indoxyle urinaire et les couleurs qui en derivent, Paris, 1903, and Zeitschr. f. physiol. Chem., **41**; Hervieux, see Bioch. Centralbl., 8, 54.

[2] Krauss, Zeitschr. f. physiol. Chem., **18**; Adrian, *ibid.*, **19**; Wang, *ibid.*, **25**; Salkowski, *ibid.*, **42**.

[3] Bouma, Zeitschr. f. physiol. Chem., **32**; Oerum, *ibid.*, **45**.

[4] Schmiedeberg, Arch. f. exp. Path. u. Pharm., **14**; G. Hoppe-Seyler, Zeitschr. f. physiol. Chem., **7** and **8**; Porcher and Hervieux, Journ. de Physiol., **7**.

[5] Gröber, Münch. med. Wochenschr., 1904; Wang, Salkowski's Festschrift, 1904; Steensma, Maly's Jahresb., **40**, 314.

Skatoxyl-sulphuric Acid, $C_9H_9NSO_4$, $C_6H_4<\begin{matrix}C.CH_3\\ \diagdown\diagup\\ NH\end{matrix}>C.O.SO_2.OH$, has not been positively prepared as a constituent of normal urine, but Otto has once prepared its alkali salt from diabetic urine. Perhaps skatoxyl occurs in normal urine as a conjugated glucuronate (Mayer and Neuberg [1]), and it is believed that the urine contains a skatol-chromogen from which red and reddish-violet coloring-matters are obtained by decomposition with strong acids and an oxidizing agent.

Skatoxyl-sulphuric acid originates, if it exists in the urine, from skatol, which is formed by putrefaction in the intestine, and which is then conjugated with sulphuric acid after oxidation into skatoxyl. That skatol introduced into the body passes partly as an ethereal-sulphuric acid into the urine has been shown by Brieger. Indol and skatol act differently, at least in dogs, indol producing a considerable amount of ethereal-sulphuric acid, while skatol gives only a small quantity (Mester [2]). Reports on this subject are at variance.

The conditions for the formation of indol and skatol by the putrefaction of proteins in the intestine are decidedly different, according to Herter, as skatol is produced by other putrefaction bacteria than indol. For example, bacillus coli communis produces indol, but only traces of skatol, while skatol is formed by certain anaerobic putrefactive bacteria. An important intermediary step in the formation of skatol is the indol acetic acid (skatol carboxylic acid, according to Salkowski) and this can also pass into the urine and is the chromogen of the urorosein, according to Herter.[3]

The potassium salt of skatoxyl-sulphuric acid is crystalline; it dissolves in water, but with difficulty in alcohol. A watery solution becomes deep violet with ferric chloride. The solution becomes red with concentrated hydrochloric acid with the separation of a red precipitate. This precipitate (skatol red) is, after washing with water, insoluble in ether but soluble in amyl alcohol. On distillation with zinc-dust the red pigment gives a strong odor of skatol.

Urines containing skatoxyl are colored dark red to violet by Jaffé's indican test even on the addition of hydrochloric acid alone; with nitric acid they are colored cherry red, and red on warming with ferric chloride and hydrochloric acid. A red coloration of the urine can also be brought about by the appearance of indigo red (indirubin) and a confusion of this pigment can also take place. Rosin [4] is of the opinion that no

[1] Otto, Pflüger's Arch., **33**; Mayer and Neuberg, Zeitschr. f. physiol. Chem., **29**.

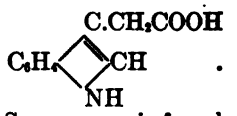
[2] Brieger, Ber. d. deutsch. chem. Gesellsch., **12**, and Zeitschr. f. physiol. Chem., **4**, 414; Mester, *ibid.*, **12**.

[3] Journ. of biol. Chem., **4**.

[4] Rosin, Virchow's Arch., **123**.

skatol chromogen exists in human urine, and that the observations made heretofore were due to a confusion of skatol red with indigo red or urorosein. It cannot be disputed that derivatives of skatol sometimes occur in human urine, and to prevent confusion with indigo red it must be borne in mind that indigo red is soluble in chloroform as well as in ether, while skatol red is insoluble in these solvents. On the contrary skatol red is soluble in amyl alcohol, and this solution shows absorption bands close to the line *D* between it and *E*, corresponding to $\lambda = 577-550$ (PORCHER and HERVIEUX [1]).

In regard to a confusion of skatol red for urorosein it must also be remarked that urorosein may also be a skatol red. The chromogen of urorosein, as HERTER has shown in a case, is identical with indol acetic acid, which passes into skatol on splitting off carbon dioxide. According to HERTER [2] urorosein is not identical with skatol red, although the investigations of STAAL, GROSSER, PORCHER and HERVIEUX [3] indicate that they are identical, and the last two investigators consider them identical, because they both have the same spectrum and the same chemical behavior.

Indol Acetic Acid (skatol-carboxylic acid), $C_{10}H_9NO_2$, 

This acid, whose occurrence in the urine was first shown by SALKOWSKI, is found in the urine in special putrefactive processes in the intestine (HERTER) and in various diseases, especially in cachectic conditions. This is of course dependent upon the fact whether indol acetic acid is the actual chromogen of urorosein, and also whether the experience obtained as to the occurrence of urorosein can be applied to the indol acetic acid. According to WECHSELMANN [4] it occurs (more correctly as urorosein) as traces in normal urine, abundantly in horse urine, and in especially large quantities in cow urine. When introduced into the animal body it appears unchanged in the urine.

This acid crystallizes in leaves which melt at 165°, and on strongly heating it yields skatol with the splitting off of carbon dioxide. The solution, acidified with hydrochloric acid, when treated with a little ferric chloride solution, becomes cherry red on boiling. With some acid and a little nitrite as well as with hydrochloric acid and chloride of lime the solution becomes red, then cloudy, and a red pigment precipitates. This pigment is soluble in amyl alcohol and gives the above-mentioned absorption bands between *D* and *E*. This red pigment is urorosein.

Urorosein is the name given by NENCKI [5] to a red pigment which occurs in the urine under the conditions mentioned under indol acetic acid. This pig-

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[1] Zeitschr. f. physiol. Chem., **45**.

[2] Journ. of biol. Chem., **4**.

[3] Staal, Zeitschr. f. physiol. Chem., **46**; Grosser, *ibid.*, **44**; Porcher and Hervieux, *ibid.*, **45**; Compt. Rend., **138**, and Journ. de Physiol., **7**.

[4] Salkowski, Zeitschr. f. physiol. Chem., **9**; Wechselmann, cited in Bioch. Centralbl., **5**, 784.

[5] Nencki and Sieber, Journ. f. prakt. Chem., (N. F.), **26**.

ment is not preformed in the urine, but is produced from its chromogen (indol acetic acid) when the urine is treated with hydrochloric acid alone. The urine becomes red. Urorosein differs from indirubin essentially by the same properties as skatol, with which, according to some, it is identical (see above).

Nephrorosein is a pigment described by V. ARNOLD [1] which is closely related to urorosein and which, like this, is produced from a chromogen when the urine is treated with nitric acid or with concentrated hydrochloric acid and a little sodium nitrite solution. Nephrorosein is soluble in amyl alcohol and gives a spectrum with a band between *b* and *F*, reaching from *b* to a little beyond the middle between *b* and *F*. It is changed by the action of light and finally gives a band between *D* and *E*, near *E*. The new pigment thus obtained is called *β-urorosein* to differentiate it from the ordinary urorosein, *α-urorosein*. The nephrorosein has not been observed in normal urines but only in certain pathological cases.

The pigment obtained by DE JAGER by precipitating the urine with HCl and formol seems to be related to urorosein and nephrorosein. According to ELLINGER and FLAMAND [2] urorosein belongs probably, like skatol-red, to the group of triindyl methane pigments prepared by them from β-indol aldehyde by boiling in acid solution. Probably the leucobase $HC.(C_8H_6N)_3$, which gives the red pigment, $HO.C:(C_8H_6N)_3$ is produced by condensation.

Aromatic Oxyacids. In the putrefaction of proteins in the intestine, *paraoxyphenyl-acetic acid* and *paraoxyphenyl-propionic acid* are formed from tyrosine as an intermediate step, and these in great part pass unchanged into the urine. The quantity of these acids is usually very small. They are increased under the same conditions as the phenols, especially in acute phosphorus poisoning, in which the increase is considerable. A small portion of these oxyacids is also combined with sulphuric acid.

Besides these two oxyacids which regularly occur in human urine we sometimes have other oxyacids in urines. To these belong *homogentisic acid* in alcaptonuria, *oxyhydroparacoumaric acid*, found by BLENDERMANN in the urine on feeding rabbits with tyrosine, *gallic acid*, which, according to BAUMANN,[3] sometimes appears in horse's urine, and *kynurenic acid* (oxyquinolincarboxylic acid), which up to the present time has been found only in dog's urine. Although all these acids do not belong to the physiological constituents of the urine, still they will be treated in connection with these.

Paraoxyphenylacetic Acid, $C_8H_8O_3$, $C_6H_4\begin{cases}OH\\CH_2.COOH\end{cases}$, and **p-Oxyphenylpropionic Acid** (Hydroparacoumaric Acid), $C_9H_{10}O_3$, $C_6H_4\begin{cases}OH\\CH_2.CH_2COOH\end{cases}$, are crystalline and are both soluble in water and in ether. The one melts at 148° C. and the other at 125° C. Both give a beautiful red coloration on being warmed with MILLON'S reagent.

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[1] Zeitschr. f. physiol. Chem., **61** and **71**.

[2] de Jager, Zeitschr. f. physiol. Chem., **61**; Ellinger and Flamand, *ibid.*, **62**.

[3] Blendermann, Zeitschr. f. physiol. Chem., **6**, 267; Baumann, *ibid.*, **6**, 193.

To detect the presence of these oxyacids proceed in the following way (BAUMANN): Warm the urine for a while on the water-bath with hydrochloric acid in order to drive off the volatile phenols. After cooling shake three times with ether, and then shake the ethereal extracts with dilute soda solution, which dissolves the oxyacids, while the residue of the phenols which are soluble in ether remains. The alkaline solution of the oxyacids is now faintly acidified with sulphuric acid, shaken again with ether, the ether removed and allowed to evaporate the residue dissolved in a little water, and the solution tested with MILLON'S reagent. The two oxyacids are best differentiated by their different melting-points. The reader is referred to other works for the method of isolating and separating these two oxyacids.

Homogentisic Acid (Dioxyphenylacetic Acid), $C_8H_8O_4$ = $C_6H_3\begin{cases}OH(1)\\OH(4)\\CH_2COOH(5)\end{cases}$. This acid, which was discovered by MARSHALL[1] and called by him *glycosuric acid*, was isolated in larger quantities by WOLKOW and BAUMANN in a case of alcaptonuria and carefully studied by them. They called it homogentisic acid because it is a homologue of gentisic acid, and they showed that the peculiar properties of so-called alcaptonuric urine in this case were due to this acid. This acid has later been found in many cases of alcaptonuria. *Glycosuric acid*, isolated from alcaptonuric urine by GEYGER,[2] seems to be identical with homogentisic acid.

The quantity of acid eliminated, which varies in most cases between 3 and 7 grams per twenty-four hours, and which is higher—14–16 grams—in exceptional cases, is increased by food rich in protein. On the ingestion of tyrosine by persons with alcaptonuria, WOLKOW and BAUMANN and EMBDEN observed a greater quantity of homogentisic acid in the urine and this has been substantiated by other observers. Since LANGSTEIN and E. MEYER showed in a case of alcaptonuria that the quantity of tyrosine in the protein, even when calculated to a maximum, was not sufficient to account for the quantity of homogentisic acid, and that therefore we must admit of another source (the phenylalanine) for the alcapton, FALTA and LANGSTEIN[3] have given a direct proof that homogentisic acid can also be formed from phenylalanine. ABDERHALDEN, BLOCH and RONA[4] have shown that in alcaptonurics the excretion of homogentisic acid is increased by the introduction of tyrosine or phenyl-

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[1] The Medical News, Philadelphia, January 8, 1887.

[2] Wolkow and Baumann, Zeitschr. f. physiol. Chem., **15**; Geyger, cited from Embden, l. c., 18. The literature can be found in Fromherz, Ueber Alkaptonurie, Inaug.-Dis. Freiburg, 1908.

[3] Langstein and Meyer, Deutsch. Arch. f. klin. Med., 78; Falta and Langstein, Zeitschr. f. physiol. Chem., 87; Falta, Der Eiweiss-Stoffwechsel bei der Alkaptonurie, Habilitationsschrift, Naumburg, a. S., 1904.

[4] Zeitschr. f. physiol. Chem., **52**.

alanine in the form of polypeptides, from dipeptides as well as tripeptides. The *p*-tyrosine and phenylalanine are quantitatively converted into homogentisic acid, in alcaptonuria (FALTA). The *m*- and *o*-tyrosine, on the contrary, are not converted, according to BLUM,[1] into homogentisic acid in alcaptonurics, and the dibromtyrosine yields just as little homogentisic acid as the bromine or iodine derivatives of protein bodies (FALTA). According to the investigations of LANGSTEIN and MEYER, and especially of FALTA, different proteins yield varying quantities of homogentisic acid in alcaptonuria, and accordingly larger amounts in proportion as the protein is rich in tyrosine and phenylalanine.

On this account the quotient H (=homogentisic acid): N (nitrogen) is variable on the introduction of different proteins. For example, with casein H: N is on an average much higher than with white of egg. In most of the cases of alcaptonuria examined the H: N was equal to 40–50: 100, and with the same alcaptonuric, when no essential change in the diet occurs, the quotient is relatively constant.

WOLKOW and BAUMANN explain the formation of homogentisic acid from tyrosine by an abnormal fermentation in the upper parts of the intestine, but this view has now been generally rejected. The observations of ABERHALDEN, BLOCH and RONA[2] that glycyl-*l*-tyrosine on subcutaneous injection causes an increased formation of homogentisic acid, disproves this theory, and indicates a formation of homogentisic acid in the tissues. This acid is also burnt in the healthy organism if not too large quantities of the acid are introduced at one time, and it is the general view that alcaptonuria is an anomaly in the protein metabolism.

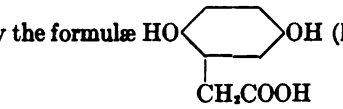
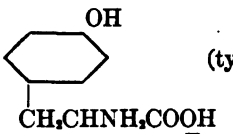
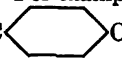
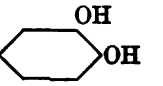
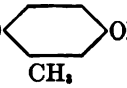
In order to understand this anomaly and the origin of the homogentisic acid we must call attention to the fact that the investigations of O. NEUBAUER and FALTA, LANGSTEIN and others[3] show that only such aromatic acids are converted, in the body, into homogentisic acid, which have a three-membered side-chain which is substituted by NH_2, OH or O in the α-position to the carboxyl group and not in the β-position. *p*-tyrosine, phenylalanine, phenyl-α-lactic acid and phenyl-pyroracemic acid are such acids. It can be admitted with FALTA that the phenylalanine in the body by deamidation is converted into phenyl-α-lactic acid, $C_6H_5.CH_2.CHOH.COOH$, from which by taking up two hydroxyl groups, dioxyphenyl-α-lactic acid (uroleucic acid), $(OH)_2C_6H_3.CH_2.CHOH.COOH$, is formed, and then from this by oxidation dioxyphenylacetic acid (homogentisic acid), $(OH)_2C_6H_3.CH_2.COOH$, is produced. Tyrosine is also supposed to undergo an analogous transformation

[1] Arch. f. exp. Path. u. Pharm., **59**.
[2] Zeitschr. f. physiol. Chem., **52**.
[3] *Ibid.*, **42**; Fromherz, l. c.

whereby a removal of the OH group in the para position must be admitted.

According to NEUBAUER,[1] on the contrary, the tyrosine, as well as the other amino-acids, is first transformed into the corresponding keto-acid, *p*-oxyphenyl pyroracemic acid, $OH.C_6H_4.CH_2.CO.COOH$, which is then oxidized into the corresponding chinol and transformed into hydroquinone pyroracemic acid, $(OH)_2C_6H_3.CH_2.CO.COOH$. The homogentisic acid is derived from this latter by the splitting off of carbon dioxide by oxidative means. Phenylalanine is either changed into phenyl pyroracemic acid or into *p*-oxyphenyl pyroracemic acid with tyrosine as intermediary body and then changed as above stated.

According to the accepted hypothesis the demolition of tyrosine and phenylalanine takes place into homogentisic acid, and the anomaly in the metabolism of alcaptonurics consists in that in these the demolition stops at this point and that the ability to rupture the benzene ring is absent, in the organism, in alcaptonuria.

The difficulties in accepting the assumption of a transformation of tyrosine into homogentisic acid due to the different positions of the hydroxyl groups in the side chain of the two bodies, as shown by the formulæ  (homogentisic acid) and  (tyrosine) do not exist now, since we have learnt of other analogous processes. For example, the oxidation, by KUMAGAI and WOLFFENSTEIN,[2] of paracresol  with potassium persulphate in acid solution. In this manner the expected 3.4 dioxytoluene  was not obtained, but instead homohydroquinone  , and hence a transference of the alkyl group must have occurred.

ABDERHALDEN[3] has also shown in healthy human beings that tyrosine may cause an elimination of homogentisic acid, as he positively detected a small quantity of homogentisic acid in the urine of a man who had taken 50 grams *l*-tyrosine per os (of which 44 grams were absorbed). In the urine of another man he could not detect either homogentisic

[1] Cited from Centralbl. f. Physiol., **23**, 76.
[2] Ber. d. d. Chem. Gesellsch., **41**.
[3] Zeitschr. f. physiol. Chem., **77**.

acid or any other intermediary product of the cleavage of tyrosine in the urine after taking 150 grams *l*-tyrosine (of which 141 grams were absorbed).

DAKIN [1] has recently opposed the above-mentioned view that in the cleavage of tyrosine and phenylalanine, homogentisic acid is always produced, and that the condition of alcaptonuria consists in an inability of the body to burn this intermediary product of metabolism. He has found that tyrosin-methyl ether, which cannot form any quinone-like intermediary product, can in cats be just as completely burnt as tyrosine, and the same is true for *p*-methylphenylalanine and for *p*-methoxyphenylalanine, which cannot form any quinone derivatives. Still these substances can be completely burnt by alcaptonurics and according to DAKIN the body in alcaptonuria has still the ability to completely burn the aromatic nucleus of tyrosine and phenylalanine when the transformation into homogentisic acid is prevented by a proper substitution in the para-groups. DAKIN therefore considers alcaptonuria as a condition in which partly the formation of an abnormal metabolic product—the homogentisic acid—takes place and where partly the ability of the body to burn this product is diminished.

GARROD,[2] who has observed several cases of alcaptonuria, has also tabulated a large number of cases of alcaptonuria which he finds in the literature, and he shows that the anomaly of the protein metabolism occurs oftener in males than in females, and also that blood relationship of the parents (first cousins) predisposes to alcaptonuria.

On fusing homogentisic acid with alkali it yields gentisic acid (hydroquinone-carboxylic acid) and hydroquinone. When introduced into the intestine of the dog a part is converted into toluhydroquinone, which is eliminated in the form of an ethereal sulphuric acid. Homogentisic acid has also been prepared synthetically by BAUMANN and FRÄNKEL, starting with gentisic aldehyde, and by NEUBAUER and FLATOW [3] from *o*-oxyphenylglyoxylic acid with hydroquinone glyoxylic acid and hydroquinone glycollic acid as intermediary bodies.

Homogentisic acid crystallizes with 1 mol. of water in large, transparent prismatic crystals, which become non-transparent at the temperature of the room with the loss of water of crystallization. They melt at 146.5–147° C., and are soluble in water, alcohol, and ether, but nearly insoluble in chloroform and benzene. Homogentisic acid is

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[1] Journ. of Biol. Chem., 8 and 9, with Wakeman, *ibid.*, 9.

[2] Med. chirurg. Transact., 1899 (where all cases up to that time are tabulated); also The Lancet, 1901 and 1902; Garrod and Hele, Journ. of Physiol., 33.

[3] Baumann and Fränkel, Zeitschr. f. physiol. Chem., 20; Neubauer and Flatow, *ibid.*, 52.

optically inactive and non-fermentable. Its watery solution has the properties of so-called alcaptonuric urine. It becomes greenish brown from the surface downward on the addition of very little caustic soda or ammonia with access of oxygen, and on shaking it quickly becomes dark brown or black.

If alcaptonuric urine or a homogentisic acid solution is treated with 10–40 per cent ordinary ammonia, a beautiful, intensive reddish-violet coloration is produced on access of air according to C. MÖRNER,[1] and two beautiful pigments, *α-* and *β-alcaptochrome*, are formed. The first, α-alcaptochrome, is crystalline and has a beautiful violet color in alkaline solution and is without fluorescence. The β-alcaptochrome is not crystalline and its alkaline solution has a more reddish color with strong fluorescence in the yellowish-red.

Homogentisic acid reduces alkaline copper solutions with even slight heat, and ammoniacal silver solutions immediately in the cold. It does not reduce alkaline bismuth solutions. It gives a lemon-colored precipitate with MILLON'S reagent, which becomes light brick-red on warming. Ferric chloride gives to the solution a blue color which soon disappears. On boiling with concentrated ferric-chloride solution an odor of quinone develops. With benzoyl chloride and caustic soda in the presence of ammonia we obtain the amide of dibenzoylhomogentisic acid, which melts at 204° C., and which can be used in the isolation of the acid from the urine, and also for its detection (ORTON and GARROD). Among the salts of this acid must be mentioned the lead salt containing water of crystallization and 34.79 per cent Pb. This salt melts at 214–215° C.

In order to prepare the acid, heat the urine to boiling, add 5 grams of lead acetate for every 100 cc., filter as soon as the lead acetate has dissolved, and allow the filtrate to stand in a cool place for twenty-four hours until it crystallizes (GARROD). The dried, powdered lead salt is suspended in ether and decomposed by H_2S. After the spontaneous evaporation of the ether the acid is obtained in almost colorless crystals (ORTON and GARROD [2]).

In regard to the quantitative estimation we proceed according to the suggestion of BAUMANN by titrating the acid with a N/10 silver solution. For details of this method the reader is referred to the works of BAUMANN, C. TH. MÖRNER, MITTELBACH, GARROD and HURTLEY. DENIGÈS [3] has suggested another method.

Uroleucic acid, $C_9H_{10}O_5$, is, according to HUPPERT, probably a dioxyphenyllactic acid, $C_6H_3(OH)_2.CH_2.CH(OH).COOH$. This acid was first prepared by KIRK from the urine of children with alcaptonuria, which also contained homogentisic acid. LANGSTEIN and MEYER [4] have also found a small amount of this acid in a case of alcaptonuria studied by them. It melts at 130–133° C. Other-

[1] Zeitschr. f. physiol. Chem., **69**.

[2] Orton and Garrod, Journ. of Physiol., **27**; Garrod, *ibid.*, **28**.

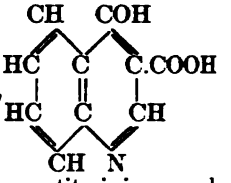
[3] Mittelbach, Deutsch. Arch. f. klin. Med., **71** (which contains the work of Baumann and Mörner); Garrod and Hurtley, Journ. of Physiol., **32**; Denigès, Chem. Centralbl., 1897, 1, 338.

[4] Huppert, Zeitschr. f. physiol. Chem., **28**; Kirk, Brit. Med. Journ., 1886 and 1888; Langstein and Meyer, l. c.

wise, in regard to its behavior with alkalies, with access of air, and also with alkaline copper solutions and ammoniacal silver solutions, and also MILLON'S reagent, it is similar to homogentisic acid.

NEUBAUER and FLATOW, who have prepared dioxyphenyl-α-lactic acid synthetically, find that this acid has entirely different properties from the so-called uroleucic acid. GARROD and HURTLEY [1] have also shown that an impure homogentisic acid with a low melting-point is easily obtained, and they suggest the possibility that the earlier reports in regard to uroleucic acid are due to an error.

Kynurenic acid (γ-oxy-β-quinolincarboxylic acid), $C_{10}H_7NO_3$,



has only been found thus far in dog's urine, but not always; its quantity is increased by meat feeding. It does not occur in the urine of cats. ELLINGER [2] has been able to show positively that tryptophane is the mother-substance of this acid. By the introduction of tryptophane in the organism he has shown the formation of a kynurenic acid not only in dogs but also in rabbits.

The acid is crystalline, does not dissolve in cold water, rather well in hot alcohol, and yields a barium salt which crystallizes in triangular, colorless plates. On heating it melts and decomposes into CO_2 and kynurin. On evaporation to dryness on the water-bath with hydrochloric acid and potassium chlorate a reddish residue is obtained which on adding ammonia becomes first brownish green and then emerald green (JAFFÉ'S reaction [3]).

Urinary Pigments and Chromogens. The yellow color of normal urine depends perhaps upon several pigments, but in greatest part upon *urochrome*. Besides this the urine seems to contain a very small quantity of *hæmatoporphyrin* as a regular constituent. *Uroerythrin* is also of frequent occurrence in normal urine, but not always. Finally, the excreted urine when exposed to the action of light regularly contains a yellow pigment, *urobilin*, which is derived from a chromogen, *urobilinogen*, by the action of light (SAILLET) and air (JAFFÉ, DISQUE [4]) and others.

Besides this chromogen, urine contains various other bodies from which coloring matters may be produced by the action of chemical agents. Humin substances (perhaps in part from the carbohydrates of the urine) may be formed by the action of acids (v. UDRÁNSZKY) without regard to the fact that such substances may sometimes originate from the reagents used, as from impure amyl

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[1] Journ. of Physiol., **36**.

[2] Ellinger, Ber. d. d. chem. Gesellsch., **37**, 1804, and Zeitschr. f. physiol. Chem., **43**. The older literature on kynurenic acid may be found in Josephsohn, Beiträge zur Kenntnis der Kynurensäure ausscheidung beim Hunde, Inaug.-Dissert., Königsberg, 1898.

[3] Zeitschr. f. physiol. Chem., **7**. In regard to kynurenic acid, see also Huppert-Neubauer, 10. Aufl., and Mendel and Jackson, Amer. Journ. of Physiol., **2**; Mendel and Schneider, *ibid.*, **5**; Camps, Zeitschr. f. physiol. Chem., **33**.

[4] Jaffé, Centralbl. f. d. med. Wissensch., 1896 and 1869, and Virchow's Arch., **47**; Disqué, Zeitschr. f. physiol. Chem., **2**; Saillet, Revue de médecine, **17**, 1897.

alcohol (v. UDRÁNSZKY [1]). To these humin bodies developed by the action of acid in normal urine when exposed to the air must be added the *urophain* of HELLER, the various *uromelanins* and other bodies described by different investigators (PLÓSZ, THUDICHUM, SCHUNCK, DOMBROWSKI [2]). Indigo blue (*uroglaucin* of HELLER, *urocyanin*, *cyanurin*, and other coloring matters of earlier investigators [3]) is split off from the indoxyl-sulphuric acid or indoxyl-glucuronic acid. Red coloring matter may be formed from the conjugated indoxyl and skatoxyl acids, and *urohodin* (HELLER), *urorubin* (PLÓSZ), *urohæmatin* (HARLEY), and perhaps also *urorosein* (NENCKI and SIEBER [4]) probably have such an origin.

We cannot discuss more in detail the different coloring matters obtained as decomposition products from normal urine. Hæmatoporphyrin has already been referred to in a previous chapter (V) and will best be described in connection with the pathological pigments. It only remains to describe urochrome, urobilin, and uroerythrin.

Urochrome is the name given by GARROD to the yellow pigment of the urine. THUDICHUM [5] had previously given the same name to a less pure pigment isolated by himself. The accounts as to the composition and properties of urochrome differ so considerably that it is questionable whether anybody has ever had this pigment in a pure form. Urochrome is free from iron, but contains nitrogen. DOMBROWSKI found 11.15 per cent nitrogen, HOHLWEG found 9.89 per cent nitrogen, and KLEMPERER found only 4.2 per cent nitrogen. According to DOMBROWSKI urochrome contains about 5 per cent sulphur, while other investigators like HOHLWEG, SALOMONSEN, and MANCINI found that it was free from sulphur.[6] According to GARROD it stands in close relation to urobilin and is transformed into urobilin by the action of "active" acetaldehyde, while RIVA [7] claims to have obtained a body similar to urochrome by the oxidation of urobilin by permanganate. This relation of the two pigments is denied by DOMBROWSKI. On the contrary it is the unanimous opinion that urochrome under certain conditions may yield the pyrrol reaction. Certain investigators such as BONDZYNSKI and DOMBROWSKI consider urochrome as a member of the oxyproteic acid group (see further on), a view which does not seem to

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[1] v. Udránszky, Zeitschr. f. physiol. Chem., **11**, **12**, and **13**.

[2] Plósz, Zeitschr. f. physiol. Chem., **8**; Thudichum, Brit. Med. Journ., **201**, and Journ. f. prakt. Chem., **104**; Schunck, cited from Huppert-Neubauer, 10. Aufl., 509; Dombrowski, Zeitschr. f. physiol. Chem., **62**.

[3] See Huppert-Neubauer, 161.

[4] In regard to this and other red pigments, see Huppert-Neubauer, 593 and 597; Nencki and Sieber, Journ. f. prakt. Chem. (2). **26**.

[5] Garrod, Proc. Roy. Soc., **55**; Thudichum, l c.

[6] Dombrowski, Zeitschr. f. physiol. Chem., **54** and **62**; Hohlweg, Bioch. Zeitschr., **13**; Salomonsen, *ibid.*, **13**; Mancini, *ibid.*, **13**; Klemperer, Berl. klin. Wochenschr., **40**.

[7] Garrod, Journ. of Physiol., **21** and **29**; Riva, cited from Huppert-Neubauer, 524.

have sufficient basis and in fact is denied by others such as WEISZ.[1] The above disputed statements as to the presence or absence of sulphur in urochrome as well as the nitrogen content of urochrome make it very probable that the preparation of pure urochrome has not thus far been accomplished.

Urochrome, as obtained thus far, is amorphous, brown, readily soluble in water and ordinary alcohol, but less soluble in absolute alcohol. It dissolves but slightly in acetic ether, amyl alcohol, and acetone, while it is insoluble in ether, chloroform, and benzene. Urochrome is precipitated by copper acetate, lead acetate, silver nitrate, mercuric acetate, phosphotungstic and phosphomolybdic acids. On saturating the urine with ammonium sulphate a great part of the urochrome remains in solution. It does not show any absorption-bands and does not fluoresce after the addition of ammonia and zinc chloride. Urochrome is very readily decomposed, by the action of acids, with the formation of brown substances.

Urochrome can be prepared according to a rather complicated method which is based upon the fact that the substance remains in great part in solution on saturating the urine with ammonium sulphate. If the proper quantity of alcohol is added to the filtrate, a clear, yellow alcoholic layer forms on the salt solution, which contains the urochrome and which can be used for the further preparation of the latter (GARROD, O. BOCCHI [2]). KLEMPERER, on the contrary, removes the pigment from the urine by means of animal charcoal, washes it with water to remove the indican and other bodies, and then extracts with alcohol and uses this alcoholic extract for the further purification according to GARROD. HOHLWEG, SALOMOSEN and MANCINI also remove the pigment from the urine, which has previously been precipitated by calcium or barium salts, by means of animal charcoal. DOMBROWSKI uses an entirely different method which is based upon the precipitation of the urochrome by copper acetate. In regard to the details of these different methods we refer to the original works.

DOMBROWSKI, BROWINSKI and DOMBROWSKI [3] have worked out a quantitative method for estimating urochrome, but its value is dependent upon a further investigation as to the purity and composition of the urochrome obtained by them. On this account the results found by these investigators will not be given. The urochrome can be quantitatively estimated, according to KLEMPERER, by a colorimetric method, using a solution of true yellow G. If 0.1 gram of this dye is dissolved in 1 liter of water and 5 cc. of this solution diluted to 50 cc. with water, then this solution has the same color and shade as a 0.1 per cent urochrome solution. The urine must be diluted with water until it has the same depth of color. The comparison is performed in vessels with parallel walls. The value of this method cannot be judged at the present time.

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[1] Dombrowski, l. c.; Bondzynski, Chem. Centralbl., 1910, Bd. II; Weisz, Bioch. Zeitschr., 30.

[2] Garrod, l. c.; Bocchi, Hofmeister's Beiträge, 11.

[3] Dombrowski, Zeitschr. f. physiol. Chem., 54, with Browinski, Bull. Acad. d. d. scien. Cracovie, 1908; Klemperer, l. c.

Urobilin is the pigment first isolated from the urine by JAFFÉ,[1] and which is characterized by its strong fluorescence and by its absorption-spectrum. Various investigators have prepared, from the urine, by different methods, pigments which differed slightly from each other but behaved essentially like JAFFÉ'S urobilin. Thus different urobilins have been suggested, such as normal, febrile, physiological, and pathological urobilins.[2] The possibility of the occurrence of different urobilins in the urine cannot be denied; but as urobilin is a readily changeable body and difficult to purify from other urinary pigments, the question as to the occurrence of different urobilins must still be considered open.

In the perfectly fresh urine of healthy human beings no urobilin occurs, as first suggested by SAILLET,[3] but only the chromogen, uribilinogen, from which the urobilin is readily formed by the action of light or by weak oxidizing agents. Pathological urines contain on the contrary preformed urobilin.

Urobilinoids, i.e., bodies which are similar to urobilin in that they fluoresce and show the same absorption spectrum have been prepared from bile-pigments (by MALY and STOKVIS) and from hæmatin or hæmatoporphyrin (by HOPPE-SEYLER, LE NOBEL, NENCKI and SIEBER, MACMUNN[4]) by reduction as well as by oxidation. According to H. FISCHER and MEYER-BETZ[5] also non-stable pyrrols, which contain a non-substituted hydrogen atom in a ring carbon atom, pass readily in the animal body into substances which give the characteristic urobilin reactions. These reactions are also given by bodies of different constitution, but which probably contain the same chromophore groups, and it is these conditions which cause the above-mentioned uncertainty as to the occurrence of different urobilins.

That urobilin is identical with the hydrobilirubin of MALY (see page 428) has been considered for a long time. In opposition to this view we find that both bodies, not to mention other small differences, have an essentially different composition. While the hydrobilirubin contains 9.22 per cent nitrogen, according to MALY, the urobilin contains only 4.09 per cent nitrogen, according to HOPKINS and GARROD, and 5.93 per cent nitrogen, according to FROMHOLDT. In the urobilin of the feces, stercobilin, which is identical with urobilin, HOPKINS and GARROD found

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[1] Centralbl. f. d. med. Wissensch., 1868 and 1869, and Virchow's Arch., **47**.

[2] See MacMunn, Proc. Roy. Soc., **31** and **35**; Ber. d. deutsch. chem. Gesellsch., **14**, and Journ. of Physiol., **6** and **10**; Bogomoloff, Maly's Jahresber., **22**; Eichholz, Journ. of Physiol., **14**; Ad. Jolles, Pflüger's Arch., **61**.

[3] Revue de médecine, **17**, 1897.

[4] Maly, Ann. d. Chem. u. Pharm., **163**; Disqué, Zeitschr. f. physiol. Chem., **2**; Stokvis, Centralbl. f. d. med. Wissensch., 1873, 211 and 449; Hoppe-Seyler, Ber. d. deutsch. chem. Gesellsch., **7**; Le Nobel, Pflüger's Arch., **40**; Nencki and Sieber, Monatshefte f. Chem., **9**, and Arch. f. exp. Path. u. Pharm., **24**; MacMunn, Proc. Roy. Soc., **31**.

[5] H. Fischer and Meyer-Betz, Zeitschr. f. physiol. Chem., **75**.

4 per cent nitrogen. Still H. FISCHER[1] has now found that the stercobilin has a lower nitrogen content because of a contamination with cholesterin or bile acids, and it is possible that also the low nitrogen content of the urine-urobilin may be caused by contamination with non-nitrogenous substances.

These possibilities have, it is sure, have not been tested; but the unequal nitrogen content of the two pigments does not positively exclude the identity of urobilin and hydrobilirubin. FISCHER and MYER-BETZ have in fact shown that the hemibilirubin, which forms about one-half of the mixture called hydrobilirubin (see page 429) is identical with the urobilinogen from human urine.

The possibility of the formation of urobilinogen and of urobilin from bile pigments is assured and many physiological as well as clinical observations[2] support the view that this transformation of the bile pigments occurs by means of putrefactive processes in the intestine. Of these observations we must mention the regular appearance in the intestinal tract of stercobilin, undoubtedly derived from the bile-pigmets; the absence of urobilin in the urine of new-born infants, as well as on the complete exclusion of bile from the intestine, and also the increased elimination of urobilin with strong intestinal putrefaction. On the other hand there are investigators who, basing their opinion on clinical observations, deny the enterogenous origin of urobilin and claim that the urobilin is derived from a transformation of the bilirubin elsewhere than in the intestine, by an oxidation of the bile-pigment or by a transformation of the blood-pigments.[3]

Urobilin or urobilinogen does not occur in the urine of all animals, and according to FROMHOLDT it is absent in the urine of rabbits. The correctness of this statement is denied by GAUTIER and ROSSO. In normal human blood they seem to be absent, while according to BIFFI[4] urobilin and urobilinogen occur sometimes in disease and in cadaver blood.

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[1] Hopkins and Garrod, Journ. of Physiol., **22**; Fromholdt, Zeitschr. f. exp. Path., u. Ther., **7**; H. Fischer, Zeitschr. f. physiol. Chem., **78**.

[2] See Fr. Müller, Schles. Gesellsch. f. vaterl. Kultur, 1892; D. Gerhardt, "Ueber Hydrobilirubin und seine Bezieh. zum Ikterus" (Inaug.-Diss., Berlin, 1889); Beck, Wien. klin. Wochenschr., 1895; Harley, Brit. Med. Journ., 1896; Fischler, Zeitschr. f. physiol. Chem., **48**.

[3] In regard to the various theories as to the formation of urobilin, see Harley, Brit. Med. Journ., 1896; A. Katz, Wien. med. Wochenschr., 1891, Nos. 28–32; Grimm, Virchow's Arch., **132**; Zoja, Conferenze cliniche italiane, Ser. 1a, **1**; Hildebrandt, Zeitschr. f. klin. Med., **59**; Biffi, Boll. d. scienc. med. di Bologna (8), anno 78, **7**; Troisier, Compt. rend. soc. biol., **66** and Tsuschija, Zeitschr. f. exp. Path. u. Ther., **7**; Fromholdt and Nersessoff, *ibid.*, **11**.

[4] Fromholdt, Zeitschr. f. physiol. Chem., **53**; Cl. Gautier and Russo, Compt. rend. soc. biol., **64**; Biffi, Folia hæmatol., **4**, and l. c. Boll., **78**.

The quantity of urobilin in the urine under physiological conditions varies widely. SAILLET found 30–130 milligrams and G. HOPPE-SEYLER 80–140 milligrams in one day's urine.

There are numerous observations on the elimination of urobilin or urobilinogen in disease, especially by JAFFÉ, DISQUÉ, GERHARDT, G. HOPPE-SEYLER,[1] and others. The quantity is increased in hemorrhage and in disease where the blood-corpuscles are destroyed, as is the case after the action of certain blood-poisons, such as antifebrin and antipyrine. It is also increased in fevers, cardiac diseases, lead colic, atrophic cirrhosis of the liver, and is especially abundant in so-called urobilin icterus.

The properties of urobilin may vary, depending upon the method of preparation and the character of the urine used; therefore only the most important properties will be given. Urobilin is amorphous, brown, reddish brown, red, or reddish yellow, depending upon the method of preparation. It dissolves readily in alcohol, amyl alcohol, and chloroform, but less readily in ether or acetic ether. It is less soluble in water, but the solubility is augmented by the presence of neutral salts. It may be completely precipitated from the urine by saturating with ammonium sulphate, especially after the addition of sulphuric acid (MÉHU[2]). It is soluble in alkalies, and is precipitated from the alkaline solution by the addition of acid. It is partly dissolved by chloroform from an acid (watery-alcoholic) solution; alkali solutions remove the urobilin from the chloroform. The neutral or faintly alkaline solutions are precipitated by certain metallic salts (zinc and lead), but not by others, such as mercuric sulphate. Urobilin is precipitated from the urine by phosphotungstic acid. It does not give GMELIN'S test for bile-pigments. It gives, on the contrary, a reaction which may be mistaken for the biuret test, by the action of copper sulphate and alkali.[3]

Neutral alcoholic urobilin solutions are, in strong concentration, brownish yellow, in great dilution yellow or rose-colored. They have a strong green fluorescence. The acid alcoholic solutions are brown, reddish yellow, or rose-red, according to concentration. They are not fluorescent, but show a faint absorption-band, λ, between b and F, which borders on F. The absorption maximum lies according to LEWIN and STENGER[4] at $\gamma = 494–497$. The alkaline solutions are brownish yellow,

[1] In regard to the literature on this subject we refer the reader to D. Gerhardt, "Ueber Hydrobilirubin und seine Beziehungen zum Ikterus" (Berlin, 1889), and also G. Hoppe-Seyler, Virchow's Arch., 124.

[2] Journ. de Pharm. et Chim., 1878, cited from Maly's Jahresber., 8.

[3] See Salkowski, Berlin. klin. Wochenschr., 1897, and Stokvis, Zeitschr. f. Biologie, 34.

[4] Pflüger's Arch., 144.

yellow, or (the ammoniacal) yellowish green, according to concentration. They show a dark band γ, which is moved somewhat toward the red end of the spectrum and lies between *E* and *F*. The absorption maximum lies at $\lambda = 506$–510. If some zinc-chloride solution is added to an ammoniacal solution of the pigment it becomes red and shows a beautiful green fluorescence and gives the same absorption bands. If a sufficiently concentrated solution of urobilin alkali is carefully acidified with sulphuric acid it becomes cloudy and shows a second band exactly at *E*, and connected with γ by a shadow (GARROD and HOPKINS, SAILLET[1]).

Urobilinogen is colorless or only faintly colored, but is very quickly changed in the air and by the action of light and is transformed into urobilin. The urobilinogen, which is identical with hemibilirubin, can be obtained as colorless prisms by solution in hot acetic-ether and treating this with ligroin, and evaporating. Urobilinogen is soluble in ether, acetic ether, amyl alcohol and in chloroform, and can in part be removed from the urine after adding sodium bicarbonate and shaking with chloroform (FISCHER and MEYER-BETZ). It can also be obtained directly from the urine or from the acidified urine by shaking with chloroform or ether, although it is less pure. In a chloroform solution of urobilin and urobilinogen, according to GRIMBERT[2], only urobilin and not urobilinogen is taken up by a sodium diphosphate solution, which is not colored red by phenolphthalein. Like urobilin, it is precipitated from the urine on saturating with ammonium sulphate. When free from urobilin it does not give any absorption bands and no fluorescence with ammonia and zinc salt. For the detection and identification of urobilinogen we make use of EHRLICH'S reagent (*p*-dimethylamino-benzaldehyde). This reagent consists of dissolving 2 grams *p*-dimethylaminobenzaldehyde in 50 cc. concentrated, fuming hydrochloric acid and diluting to 100 cc. with water. To 10 cc. of the urine add 1 cc. of the reagent and thoroughly shake. According to the amount of urobilinogen, the solution becomes pink colored or intensely red, and in the spectrum we find a band between *D* and *E*. The red color can be taken up by amyl alcohol. Urobilin does not give this reaction, which is common to certain hæmatin and pyrrol derivatives.

The *preparation of urobilin* from the urine can be done according to the original method of JAFFÉ, or according to the method suggested by MÉHU, which has been modified somewhat by GARROD and HOPKINS[3] (precipitation with ammonium sulphate) or according to CHARNAS' suggestion.

According to CHARNAS[4] the preparation is best from urobilinogen,

[1] Garrod and Hopkins, Journ. of Physiol., **20**; Saillet, l. c.

[2] Fischer and Meyer-Betz, l. c.; Grimbert, Compt. rend. soc. biol., **70**.

[3] Jaffé, l. c.; Méhu, l. c.; Garrod and Hopkins, Journ. of Physiol., **20**.

[4] Charnas, Bioch. Zeitschr., **20**.

and if the urine contains urobilin, it is first allowed to undergo alkaline fermentation, when the urobilin is converted into urobilinogen. The urine is acidified with tartaric acid and extracted with ether. The foreign pigments are precipitated from the ethereal solution by petroleum ether; the ether solution is washed with water, evaporated, and the residue allowed to stand with water for several hours at 38° C., when the urobilinogen is transformed into urobilin. The urobilin can now be precipitated with ammonium sulphate, and the dried precipitate extracted with absolute alcohol. This urobilin has about three times as much extinction ability as MALY'S urobilin (hydrobilirubin). Other methods of preparation have been suggested.

The *urobilinogen* is *prepared* by shaking the urine, directly after adding sodium bicarbonate, with chloroform (FISCHER and MEYER-BETZ). In regard to details we must refer to the original publication.

The detection of urobilin can sometimes be done directly on the urine. Otherwise the urine is shaken with ether, amyl alcohol or chloroform and these solutions tested. According to SCHLESINGER[1] the urine can also be precipitated by an equal volume of a saturated solution of zinc acetate in alcohol and the filtrate directly tested for the fluorescence and absorption. GRIMBERT[2] has suggested a method for the separate testing for urobilin and urobilinogen by using the chloroform, after shaking the urine therewith. For the detection of urobilin we always make use of the color of the acid or alkaline solutions, the absorption spectrum and the beautiful fluorescence of the ammoniacal solution containing zinc chloride. For the detection of urobilinogen we make use of EHRLICH'S reagent, and the property of the colorless solution of being changed into urobilin in the air and light.

In the quantitative estimation of urobilin we proceed as follows, according to G. HOPPE-SEYLER:[3] 100 cc. of the urine are acidified with sulphuric acid and saturated with ammonium sulphate. The precipitate is collected on a filter after some time, washed with a saturated solution of ammonium sulphate, and repeatedly extracted with equal parts of alcohol and chloroform after pressing. The filtered solution is treated with water in a separatory funnel until the chloroform separates well and becomes clear. The chloroform solution is evaporated on the water-bath in a weighed beaker, the residue dried at 100° C., and then extracted with ether. The ethereal extract is filtered, the residue on the filter dissolved in alcohol, and transferred to the beaker and evaporated, then dried and weighed. According to this method G. HOPPE-SEYLER found 0.08–0.14 gram of urobilin in one day's urine of a healthy person, or an average of 0.123 gram.

The urobilin can also be determined according to the method suggested by CHARNAS for its preparation and urobilin can also be determined spectroscopically by the method suggested by SAILLET.[4] Further details will be found in the original publications and in larger handbooks.

The quantitative estimation of urobilinogen can be accomplished spectroscopically by means of EHRLICH'S reagent, as suggested by CHARNAS.

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[1] Deutsch. med. Wochenschr., 1903.

[2] Compt. rend. soc. biol., **70**.

[3] Virchow's Arch., **124**.

[4] Charnas, l. c.; Saillet, l. c.; see also Tsuschija, Zeitschr. f. exp. Path. u. Ther., **7**.

Uroerythrin is the pigment which often gives the beautiful red color to the urinary sediments (*sedimentum lateritium*). It also frequently occurs although only in very small quantities, dissolved in normal urines. The quantity is increased after great muscular activity, after profuse perspiration, immoderate eating, or partaking of alcoholic drinks, as well as after digestive disturbances, fevers, circulatory disturbances of the liver, and in many other pathological conditions.

Uroerythrin, which has been especially studied by ZOJA, RIVA, and GARROD,[1] has a pink color, is amorphous, and is very quickly destroyed by light, especially when in solution. The best solvent is amyl alcohol; acetic ether is not so good, and alcohol, chloroform, and water are even less valuable. The very dilute solutions show a pink color; but on greater concentration they become reddish orange or bright red. They do not fluoresce either directly or after the addition of an ammoniacal solution of zinc chloride; but they have a strong absorption, beginning in the middle between *D* and *E* and extending to about *F*, and consisting of two bands which are connected by a shadow between *E* and *b*. Concentrated sulphuric acid colors a uroerythrin solution a beautiful carmine red; hydrochloric acid gives a pink color. Alkalies make its solution grass green, and often a play of colors from pink to purple and blue is observed. PORCHER and HERVIEUX[2] claim that uroerythrin is a skatol pigment.

In preparing uroerythrin according to GARROD, the sediment is dissolved in water at a gentle heat and saturated with ammonium chloride, which precipitates the pigment with the ammonium urate. This is purified by repeated solution in water and precipitation with ammonium chloride until all the urobilin is removed. The precipitate is finally extracted on the filter in the dark with warm water, filtered, then diluted with water, any hæmatoporphyrin remaining being removed by shaking with chloroform; the precipitate is then faintly acidified with acetic acid and shaken with chloroform, which takes up the uroerythrin. The chloroform is evaporated in the dark at a gentle heat.

Volatile fatty acids, such as formic acid, acetic acid, and perhaps also butyric acid, occur under normal conditions in human urine (v. JAKSCH), also in that of dogs and herbivora (SCHOTTEN). The acids poorest in carbon, such as formic acid and acetic acid, are more stable in the body than those richer in carbon, and therefore the relatively greater part of these pass unchanged into the urine (SCHOTTEN). Normal human urine contains besides these bodies others which yield acetic acid when oxidized by potassium dichromate and sulphuric acid (v. JAKSCH). The quantity of volatile fatty acids in normal urine calculated as acetic acid is, according to v. JAKSCH, 0.008–0.009 gram per twenty-four hours; according to v. ROKITANSKY, 0.054 gram; and according to MAGNUS-LEVY 0.060 gram. The quantity is increased by exclusively farinaceous food (ROKITANSKY), in fever and in certain diseases, while in others it is diminished (v. JAKSCH, ROSENFELD). Large amounts of volatile fatty acids are produced in the alkaline fermentation of the urine, and the quantity is 6–15 times as large as in

[1] Zoja, Arch. ital. di clinica med., 1893, and Centralbl. f. d. med. Wissensch., 1892; Riva, Gaz. med. di Torino, Anno 43, cited from Maly's Jahresber., 24; Garrod, Journ. of Physiol., 17 and 21.

[2] Journ. de Physiol., 7.

normal urine (SALKOWSKI).[1] *Non-volatile fatty acids* have been detected as normal constituents of urine by K. MÖRNER and HYBBINETTE.[2]

Paralactic Acid. It is claimed that this acid occurs in the urine of healthy persons after very fatiguing marches (COLASANTI and MOSCATELLI). It is found in larger amounts in the urine in acute phosphorus-poisoning or acute yellow atrophy of the liver (SCHULTZEN and RIESS), in pregnancy (UNDERHILL), and especially abundant in eclampsia (ZWEIFEL and others). According to the investigations of HOPPE-SEYLER, ARAKI, and V. TERRAY, lactic acid passes into the urine as soon as the supply of oxygen is decreased in any way, and this probably explains the occurrence of lactic acid in the urine after epileptic attacks (INOUYE and SAIKI). MINKOWSKI [3] has shown that lactic acid occurs in the urine in large quantities on the extirpation of the liver of birds.

Glycerophosphoric acid occurs as traces in the urine,[4] and it is probably a decomposition product of lecithin. The occurrence of *succinic acid* in normal urine is a subject of discussion.

Carbohydrates and Reducing Substances in the Urine. The occurrence of *glucose*, as traces, in normal urine is highly probable, as the investigations of BRÜCKE, ABELES, and V. UDRÁNSZKY show. The last investigator has also shown the habitual occurrence of carbohydrates in the urine, and their presence has been positively proven by the investigations of BAUMANN and WEDENSKI, and especially by BAISCH. Besides glucose normal urine contains, according to BAISCH, another not well-studied variety of sugar, according to LEMAIRE, probably isomaltose, and besides this a dextrin-like carbohydrate (animal gum), as shown by LANDWEHR, WEDENSKI, and BAISCH. The quantity of carbohydrates eliminated under normal conditions in the twenty-four hours' urine and determined by the benzoylation method, which is perhaps not sufficiently trustworthy, varies considerably between 1.5 and 5.09 grams.[5]

The precipitate obtained from concentrated urine by the aid of alcohol and whose nitrogen (colloidal nitrogen according to SALKOWSKI) in normal urine amounts to 2.34–4.08 per cent of the total nitrogen, and in pathological urines to 8–9 per cent, and in a case of acute yellow atrophy of the liver to 21.8 per cent contains, SALKOWSKI [6] claims, a nitrogenous carbohydrate which has strong

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[1] v. Jaksch, Zeitschr. f. physiol. Chem., **10**; Schotten, *ibid.*, **7**; Rokitansky, Wien. med. Jahrbuch, 1887; Salkowski, Zeitschr. f. physiol. Chem., **13**; Magnus-Levy, Salkowski's Festschrift, 1904; Rosenfeld, Deutsch, med. Wochenschr., **29**.

[2] Skand. Arch. f. Physiol., **7**.

[3] Colasanti and Moscatelli, Moleschott's Untersuch., **14**; Schultzen and Reiss, Chem. Centralbl., 1869; Underhill, Journ. of biol. Chem., **2**; Zweifel, Arch. f. Gynäkol., **76**; Araki, Zeitschr. f. physiol. Chem., **15**, **16**, **17**, **19**. See also Irisawa, *ibid.*, **17**; v. Terray, Pflüger's Arch., **65**; Schütz, Zeitschr. f. physiol. Chem., **19**; Inouye and Saiki, *ibid.*, **37**; Minkowski, Arch. f. exp. Path. u. Pharm., **21** and **31**.

[4] See Pasqualis, Maly's Jahresber., **24**.

[5] Lemaire, Zeitschr. f. physiol. Chem., **21**; Baisch, *ibid.*, **18**, **19**, and **20**. In these as well as in Treupel, *ibid.*, **16**, the works of other investigators are cited. See also v. Alfthan, Deutsch. med. Wochenschr., **26**.

[6] Berlin. klin. Wochenschr., 1905. In regard to urinary colloids see also Lichtwitz, Zeitschr. f. physiol. Chem., **61** and **72**, with Rosenbach, *ibid.*, **61**.

reducing action upon alkaline copper solutions after cleavage with hydrochloric acid.

Besides traces of sugar and the reducing substances previously mentioned, uric acid and creatinine, the urine contains still other bodies of this character. These latter are partly conjugated compounds of *glucuronic acid*, $C_6N_{10}O_7$, which is closely allied to sugar. The reducing power of normal urine corresponds, according to various investigators, to 1.5–5.96 p. m. glucose. That portion of the reduction belonging to glucose alone is equal to 0.1–0.6 p. m. LAVESON[1] believes that of the total reduction 17.8 per cent is due to sugar, 26.3 per cent to creatinine, 7.8 per cent to uric acid, and the remainder, nearly 50 per cent, is caused by chiefly unknown bodies.

Conjugated glucuronates occur, as indicated by FLÜCKIGER and first positively shown by MAYER and NEUBERG, in an exact manner, in very small amounts in normal urine. They occur chiefly as phenol- and only very small amounts of indoxyl- or skatoxylglucuronates. The quantity of glucuronic acid obtained from the conjugated glucuronates is estimated as 0.04 p. m. by MAYER and NEUBERG, and by C. TOLLENS and FR. STERN,[2] on the contrary it was found to be 2.5 p. m. or 0.37 gram per day. Besides these conjugated glucuronates perhaps the urine sometimes contains the urea glucuronic acid, the ureidoglucuronic acid prepared synthetically by NEUBERG and NIEMANN.[3]

Very large amounts of these conjugated glucuronates occur in the urine, on the other hand, after partaking of various therapeutic agents and other substances, such as chloral hydrate, camphor, naphthol, borneol, turpentine, morphine, and many other substances. The elimination of glucuronic acid may be markedly increased in severe disturbances of the respiration, severe dyspnœa, in diabetes mellitus, and by the direct introduction of large amounts of glucose. According to P. MAYER, in the oxidation of glucose a part of it forms glucuronic acid, hence it is to be expected that the glucuronic acid can in part be derived from the glucose. As a conjugation of the glucuronic acid with other bodies, such as aromatic atomic complexes, prevents the combustion of this acid in the animal body, it ought to follow that after the introduction of such an atomic complex in the body during a glycosuria a corresponding reduction of the glucose elimination would take place with the increased excretion of conjugated glucuronates. In order to prove this possibility, O. LOEWI[4] fed dogs with camphor during phlorhizin

[1] Flückiger, Zeitschr. f. physiol. Chem., **9**; Laveson, Bioch. Zeitschr., **4**.

[2] Flückiger, l. c.; Mayer and Neuberg, Zeitschr. f. physiol. Chem., **29**; Tollens and Stern, Zeitschr. f. physiol. Chem., **67**.

[3] Zeitschr. f. physiol. Chem., **44**.

[4] Arch. f. exp. Path. u. Pharm., **47**.

diabetes and found that the above expectation was not realized. Although large quantities of campho-glucuronic acid were excreted, the sugar excretion was only slightly diminished and not in proportion to the quantity of conjugated glucuronate excreted. These negative results are contradicted by the positive results obtained by PAUL MAYER.[1] Rabbits normally convert almost all the camphor introduced into conjugated glucuronic acid. MAYER claims that if we allow a rabbit to starve several days, the animal becomes so poor in the mother-substance (glycogen) yielding the glucuronic acid that the introduction of camphor only brings about an elimination of small quantities of glucuronic acid. By the simultaneous administration of camphor and glucose while starvation is going on, the elimination of glucuronic acid rises again to the same height as it was before the starvation period. This shows that the sugar had conjugated itself with the camphor as glucuronic acid. HILDEBRANDT[2] has also made experiments showing that glucuronic acid can very likely be formed from sugar. The observations of MAYER are not substantiated by the recent investigations of FENYVESSY,[3] and the observers do not agree on this question.

The conjugated glucuronic acids are formed, based upon the investigations of SUNDWIK, FISCHER and PILOTY,[4] by a combination taking place first between the conjugator and the glucose by means of the aldehyde group, and then the end alcohol group, CH_2OH, is oxidized to COOH. The conjugated glucuronic acids, at least in most cases, seem to be constructed after the glucoside type, a view which has received further support by the synthesis of phenolglucuronic acid and euxanthonglucuronic acids by NEUBERG and NEIMANN.[5] Based upon their cleavage (as far as they have been investigated) by kephir lactase and emulsin, but not by yeast lactase (NEUBERG and WOHLGEMUTH[6]), the conjugated glucuronic acids must belong to the β-series of glucosides. We also know of certain conjugated glucuronates that are constructed upon the ester type, namely, the dimethylaminobenzoicglucuronate, discovered by JAFFÉ and also the benzoicglucuronic acid, after feeding benzoic acid (MAGNUS-LEVY).[7]

According to the body with which they are conjugated the glucuronates vary in behavior. On taking up water they split into glucuronic acid

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[1] Zeitschr. f. klin. Med., 47.

[2] Arch. f. exp. Path. u. Pharm., 44.

[3] See Maly's Jahresber., 34.

[4] E. Sundwik, Akademische Abhandlung Helsingfors, 1886; see also Maly's Jahresber., 16, 76; Fischer and Piloty, Ber. d. d. chem. Gesellsch., 24.

[5] Zeitschr. f. physiol. Chem., 44.

[6] See Neuberg, Ergebnisse der Physiologie, Bd. 3, Abt. 1, 444.

[7] Jaffé, Zeitschr. f. physiol. Chem., 43; Magnus-Levy, Bioch. Zeitschr., 6.

and the conjugated group and this is brought about by boiling with a dilute mineral acid. They are precipitated by basic lead acetate or by basic lead acetate and ammonia. Most of the conjugated glucuronic acids do not have a direct reducing action but are reducing after hydrolysis. Certain of them, and to this group belong especially those acids of the ester type, reduce copper oxide and certain other metallic oxides in alkaline solution directly, and hence cause errors in the investigations of the urine for sugar. The conjugated acids of the glucoside type rotate the plane of polarized light to the left, while the glucuronic acid itself is dextrorotatory. The conjugated acids of the ester type, which as a rule are less stable, rotate the ray of polarized light to the right. As the detection of conjugated glucuronic acids is connected with the tests for sugar in the urine, we will treat of this in connection with these tests.

Organic combinations containing sulphur of unknown kind, which may in small part consist of *sulphocyanides*, 0.04 (GSCHEIDLEN) to 0.11 p. m. (I. MUNK),[1] *cystine* or bodies related to it, *taurine derivatives*, *chondroitin-sulphuric acid* and *protein bodies*, but in greater part are made up of *antoxyproteic acid*, *oxyproteic acid*, *alloxyproteic acid*, and *uroferric acid*, are found in human as well as in animal urines. The sulphur of these mostly unknown combinations has been called "neutral," to differentiate it from the "acid" sulphur of the sulphate and ethereal-sulphuric acid (SALKOWSKI [2]). The neutral sulphur in normal urine is 13–24 per cent of the total sulphur.[3] In anæmia, cachetic conditions, pulmonary tuberculosis and especially in carcinoma the quantity is greatly increased (WEISS). In general it can be said that the quantity is increased by an increased catabolism of protein and therefore an increase in the neutral sulphur has been found in starvation (FR. MÜLLER), with insufficient oxygen supply (REALE and BOERI, HARNACK and KLEINE) and after chloroform narcosis (KAST and MESTER). After the introduction of free sulphur the quantity of neutral sulphur is increased, according to PRESCH and YVON and to MAILLARD.[4] The quantity of neutral sulphur varies, according to BENEDICT, within rather narrow limits and especially, according to FOLIN, it is dependent to a less degree than the sulphate excretion upon the extent of the protein metabolism. The relation between the neutral and acid sulphur depends in the first place

[1] Gscheidlen, Pflüger's Arch., **14**; Munk, Virchow's Arch., **69**.

[2] *Ibid.*, **58**, and Zeitschr. f. physiol. Chem., **9**.

[3] Salkowski, l. c.; Stadthagen, Virchow's Arch., **100**; Lépine, Compt. Rend., **91** and **97**; Harnack and Kleine, Zeitschr. f. Biologie, **37**; Mor. Weiss, Bioch. Zeitschr., **27**.

[4] Weiss, l. c.; Fr. Müller, Berl. klin. Wochenschr., 1887; Reale and Boeri, Maly's Jahresber., **24**; Harnack and Kleine, l. c.; Kast and Mester, Zeitschr. f. klin. Med., **18**; Presch., Virchow's Arch., **119**; Yvon, Arch. de Physiol. (5), **10**; Maillard, Compt. Rend., **152**.

upon the extent of the sulphuric-acid excretion. According to HARNACK and KLEINE,[1] the relation of the oxidized sulphur to the total sulphur changes always in the same way as the relation of the nitrogen of the urea to the total nitrogen. The more unoxidized sulphur is eliminated the more abundant are the nitrogen compounds, not urea, in the urine—a statement which coincides with recent observations showing that the neutral sulphur originates chiefly from the different proteic acids, and the uroferric acid.

According to LÉPINE, a part of the neutral sulphur is more readily oxidized (directly with chlorine or bromine) into sulphuric acid than the other, which is only converted into sulphuric acid after fusing with potash and saltpeter. The investigations of W. SMITH [2] show that it is probable that the difficultly oxidizable part of the neutral sulphur occurs as sulpho-acids. An increased elimination of neutral sulphur has been observed in various diseases, such as pneumonia, cystinuria, and especially where the flow of bile into the intestine is prevented.

The total quantity of sulphur in the urine is determined by fusing the solid urinary residue with saltpeter and caustic alkali or sodium peroxide, or by oxidation with nitric acid.[3] The quantity of neutral sulphur is determined as the difference between the total sulphur and the sulphur of the sulphate and ethereal-sulphuric acids. The readily oxidizable part of the neutral sulphur is determined by oxidation with bromine or potassium chlorate and hydrochloric acid (LÉPINE, JEROME [4]).

Sulphuretted hydrogen occurs in the urine only under abnormal conditions or as a decomposition product. This compound may be produced from the neutral sulphur of the organic substances of the urine by the action of certain bacteria (FR. MÜLLER, SALKOWSKI [5]). Other investigators have given *hyposulphites* as the source of the sulphuretted hydrogen. The occurrence of hyposulphites in normal human urine, which is asserted by HEFFTER, is disputed by SALKOWSKI and PRESCH.[6] Hyposulphites occur constantly in cat's urine and, as a rule, also in dog's urine.

Antoxyproteic acid is a nitrogenous acid containing sulphur which BONDZYNSKI, DOMBROWSKI, and PANEK [7] have isolated from human urine. The composition of the acid was: C 43.21, H 4.91, N 24.4, S 0.61, and O 26.33 per cent. A part of the sulphur can be split off by alkali. This acid is soluble in water, is dextrorotatory, and is only precipitated from concentrated solution by phosphotungstic acid. It does not give the protein color reactions, but gives EHRLICH'S diazo reaction (see below).

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[1] Benedict, Zeitschr. f. klin. Med., **36**; Harnack and Kleine, l. c.; Folin, Amer. Journ. of Physiol., **18**.

[2] Lépine, l. c.; Smith, Zeitschr. f. physiol. Chem., **17**.

[3] See Abderhalden and Funk, Zeitschr. f. physiol. Chem., **58** and **59**, which also cites other methods. See S. R. Benedict, Journ. of biol. Chem., **6** and **8**; Denis, *ibid.*, **8**; Gill and Grindley, Journ. Amer. Chem. Soc., **31**; Folin, *ibid.*, **31**.

[4] Jerome, Pflüger's Arch., **60**.

[5] Fr. Müller, Berlin. klin. Wochenschr., 1887; Salkowski, *ibid.*, 1888.

[6] Heffter, Pflüger's Arch., **38**; Salkowski, *ibid.*, **39**; Presch, Virchow's Arch., **119**.

[7] Zeitschr. f. physiol. Chem., **46**.

The salts with the alkalies, barium, calcium, and silver are soluble in water, and of these salts that with barium and, to a still higher degree, the silver salt are soluble with difficulty in alcohol. The free acid and its salts are precipitated by mercuric nitrate and acetate, and by this last reagent even from solutions strongly acidified with acetic acid. Basic lead acetate does not precipitate the pure acid.

Oxyproteic acid is the name given by BONDZYNSKI and GOTTLIEB[1] to a nitrogenous acid containing sulphur, and which they prepared from human urine, which has recently been further studied by BONDZYNSKI, DOMBROWSKI and PANEK. This acid contained C 39.62, H 5.64, N 18.08, S 1.12, and O 35.54 per cent, and also contains sulphur which could be split off. On cleavage it yields no tyrosine, nor does it give EHRLICH'S diazo reaction, the xanthoproteic nor the biuret reaction. It gives a faint indication of a MILLON reaction and is not precipitated by phosphotungstic acid, hence it leads to an error in the PFLÜGER-BOHLAND method for estimating urea. The acid soluble in water is precipitated by mercuric nitrate and acetate in neutral solutions, but is not precipitated by basic lead acetate. The salts of this acid are readily soluble in water and more soluble in alcohol than the corresponding salts of antoxyproteic acid.

The acid which is found in large quantities, especially in the urine of dogs poisoned with phosphorus (BONDZYNSKI and GOTTLIEB), is considered like the preceding acid as an intermediary oxidation product of the proteins, and oxyproteic acid seems to represent a higher state of oxidation or a demolition of the proteins than the antoxyproteic acid.

The acid called *uroproteic acid* by CLOETTA is probably a mixture of several bodies, according to the recent investigations of BONDZYNSKI, DOMBROWSKI, and PANEK. The same applies also to the barium oxyproteate prepared by PREGL[2] from the urine.

Alloxyproteic acid is a third acid related to the above, which was first isolated by BONDZYNSKI and PANEK[3] from the urine and then carefully studied with DOMBROWSKI. The composition is: C 41.33, H 5.70, N 13.55, S 2.19, and O 37.23 per cent, based upon new investigations. The free acid is soluble in water. It gives neither the biuret reaction nor EHRLICH'S reaction, and is not precipitated by phosphotungstic acid. Differing from the other acids, it is precipitated by basic lead acetate, and its salts are only slightly soluble in alcohol. According to

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[1] Centralbl. f. d. med. Wissensch., 1897, No. 33.

[2] Cloetta, Arch. f. exp. Path. u. Pharm., **40**; Pregl, Pflüger's Arch., **75**.

[3] Ber. d. d. chem. Gesellsch., **35**.

LIEBERMANN [1] this acid is not a unit substance, and contains a part of its sulphur as ethereal sulphuric acid, and it also contains uroferric acid.

The urochrome, which has been specially studied by DOMBROWSKI,[2] is considered by him and BONDZYNSKI as belonging to the oxyproteic acid group. I contains about 5 per cent sulphur, is precipitated by copper acetate and yields melanin-like substances on its decomposition. No positive proofs are at hand in regard to the purity of this urochrome and the reports as to its composition, which are very contradictory, do not exclude the possibility that this is a mixture of a yellow pigment with another substance (see page 741).

BROWINSKI and DOMBROWSKI [3] have carried out investigations on the nitrogen titratable with formol on the oxyproteic acids before and after acid hydrolysis. They found that the antoxyproteic acid and the oxyproteic acid did not contain any nitrogen split off as NH_3 by MgO before hydrolysis, while the alloxyproteic acid as well as the urochrome yielded about 3 per cent of the total nitrogen in this form. After acid hydrolysis all gave about the same quantity of ammonia. The two first-mentioned acids, especially oxyproteic acid, were before hydrolysis considerably richer in amino groups, titratable with formol, than the others. This indicates that these two acids are produced from the proteins by a deeper cleavage than is the alloxyproteic acid. The large amount of free amino groups, which occur especially in the oxyproteic acid and which amount to 38.8 per cent of the total nitrogen, is nevertheless remarkable.

The preparation of the three above-mentioned acids is based in part upon the fact that alloxyproteic acid alone is precipitated by basic lead acetate and that the two other acids can be precipitated from the filtrate by mercuric acetate, the antoxyproteic acid in acetic acid solution and the oxyproteic acid in neutral solution. The preparation is nevertheless very tedious and complicated and therefore we must refer to the original works for details.

Uroferric acid is an acid isolated by THIELE [4] from the urine, according to SIEGFRIED'S method for preparing pure peptone. It also contains sulphur, 3.46 per cent, and has the formula $C_{35}H_{56}N_8SO_{19}$. The acid forms a white powder which is readily soluble in water, saturated ammonium-sulphate solution, and methyl alcohol. It is soluble with difficulty in absolute alcohol, insoluble in benzene, chloroform, ether, and acetic ether. About one-half of the sulphur can be split off as sulphuric acid on boiling with hydrochloric acid. The acid gives neither the biuret test nor MILLON'S or ADAMKIEWICZ'S reactions. It is precipitated by mercuric nitrate and sulphate, and also by phosphotungstic acid. This acid is hexabasic, and its specific rotation at 18° C. $(\alpha)_D = -32.5°$. On cleavage it yields melanine substances, sulphuric acid, aspartic acid, but no hexone bases. The existence of this acid is disputed by BONDZYNSKI, DOMBROWSKI and PANEK. The investigations of GINSBERG also contradict the occurrence of such an acid, because no sulphuric acid could be split off from the mixture of the oxyproteic acids by hydrolysis.

Methods for the quantitative estimation of the total oxyproteic acids have been suggested by GINSBERG and by GAWINSKI.[5] Accord-

[1] Zeitschr. f. physiol. Chem., **52**.

[2] *Ibid.*, **46** and **62**.

[3] *Ibid.*, **77**.

[4] Zeitschr. f. physiol. Chem., **37**.

[5] Ginsberg, Hofmeister's Beiträge, **10**; Gawinski, Zeitschr. f. physiol. Chem., **58**.

ing to their determinations, in man with a mixed diet, the nitrogen of the oxyproteic acids represented 3–6.8 per cent of the total nitrogen, and with a milk diet it sinks to about one-half of this (GAWINSKI). In dogs it amounts to 2 per cent of the total nitrogen (GINSBERG). In disease it may rise, and in typhoid cases it may rise to 14.69 per cent of the total nitrogen (GAWINSKI). In phosphorus poisoning this nitrogen fraction is also markedly increased according to several observations. The oxyproteic acids are considered, as above remarked, as intermediary products of the protein metabolism, and GAWINSKI holds that the elimination of their nitrogen runs parallel with the elimination of neutral sulphur, so that this latter may serve as an approximate measure of the elimination of these acids.

ABDERHALDEN and PREGL [1] have shown that human urine normally contains compounds which stand, perhaps, in close relation to the polypeptides, and which on hydrolysis with acids yield at least a part of the moities existing in the protein molecule. In the case investigated they obtained abundant glycocoll, also leucine, alanine, glutamic acid, phenylalanine, and probably also aspartic acid. The relation between these polypeptide-like bodies and the above-mentioned proteic acids and uroferric acid has not been investigated.

HENRIQUES and SÖRENSEN [2] have given further proof for the occurrence of nitrogen in peptide combinations in the urine. They have shown by formol titration that in normal urine amino-acid nitrogen occurs. It must be remarked that they consider as amino-acid nitrogen not only the nitrogen occurring as amino-acids but also the urine nitrogen directly titratable with formol, therefore also the titratable amino-nitrogen in the oxyproteic acids, polypeptides or more complicated protein derivatives. They have further shown that after boiling with acid that the quantity of titratable nitrogen increases, and this increase which in man may be 8.9–28.3 per cent of the amino-acid nitrogen, they consider as peptide-like nitrogen. We have abundant literature [3] on the ways and means of carrying out the formol titration in urines, considering the presence of ammonia.

Amino-acids may, when they are introduced into the body in large amounts, also pass in part into the urine. This has been shown for *r*-alanine by R. HIRSCH in the dog, and by PLAUT and REESE in dog and man, and for *r*-leucine by ABDERHALDEN and SAMUELY [4] in rabbits and by others using different amino-acids. EMBDEN and REESE, FORSSNER, ABDERHALDEN and SCHITTENHELM, SAMUELY, EMBDEN and MARX [5] were able, by means of the naphthalene sulphochloride method to detect glycocoll in normal human urine, and this glycocoll must occur in the urine in a combination which is readily split by alkali. Although

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[1] Zeitschr. f. physiol. Chem., **46**.

[2] Henriques, Zeitschr. f. physiol. Chem., **60**; Henriques and Sörensen, *ibid.*, **63** and **64**.

[3] See Henriques and Sörensen, l. c.; Malfatti, Zeitschr. f. physiol. Chem., **61** and **66**; de Jager, *ibid.*, **62** and **65**; Fry and Gigon, Bioch. Zeitschr., **22**.

[4] R. Hirsch, Zeitschr. f. exp. Path. u. Therap., **1**; Plaut and Reese, Hofmeister's Beiträge, **7**; Abderhalden and Samuely, Zeitschr. f. physiol. Chem., **47**.

[5] Forssner, Zeitschr. f. physiol. Chem., **47**; Abderhalden and Schittenhelm, *ibid.*, **47**; Samuely, *ibid.*, **47**; Embden and Reese, Hofmeister's Beiträge, **7**, with Marx, *ibid.*, **11**, which also cites the rather conflicting deductions of Neuberg and Wolgemuth and of Hirschstein.

there have been numerous investigations, no amino-acids besides glycocoll could be detected in normal human urine, while, on the contrary, in pathological conditions other amino-acids have been found several times. The amino-acid fraction of the urine seems to be increased in starvation and in high altitudes (LOEWY [1]). The conclusions of various investigators [2] in regard to the behavior of amino-acids in diseases such as gout, disagree.

Non-dialyzable substances, the so-called *adialyzable bodies*, or bodies that dialyze with difficulty, also occur in the urine. They consist in part of chondroitin-sulphuric acid whose daily amount, according to PONS, is 0.08–0.09 gram, and also of nucleic acid, mucoids, the colloidal nitrogenous bodies (see SALKOWSKI, page 749) and unknown bodies. SASAKI found 0.218–0.68 gram of such bodies per liter of normal urine, and EBBECKE found 1.44 grams in men. In pregnant women SAVARÈ found somewhat higher results (0.6 gram per liter) than in non-pregnant women (0.4 gram). The quantity is increased in fevers, in pneumonia (EBBECKE), in nephritis, and especially in eclampsia, where SAVARÈ [3] indeed in one case found 13.84 grams per liter. The adialyzable bodies occurring in eclampsia are toxic.

Organic combinations containing phosphorus such as glycerophosphoric acid, phosphocarnic acid (ROCKWOOD), etc., which yield phosphoric acid on fusing with saltpeter and caustic alkali, are also found in urine (LÉPINE and EYMONNET, OERTEL). With a total elimination of about 2.0 grams total P_2O_5, OERTEL found on an average about 0.05 gram P_2O_5 as phosphorus in organic combination. According to KONDO the quantity of organic phosphorus is increased by taking phosphatides and nucleins but not to the same extent as the quantity of phosphoric acid. According to SYMMERS [4] the organic combined phosphorus may in many pathological conditions be 25–50 per cent of the total phosphoric acid. In lymphatic leucæmia, and especially in degenerative diseases of the nervous system, the quantity may increase.

Enzymes of various kinds have been isolated from the urine. Among these may be mentioned *pepsin*, *diastatic enzyme* and *lipase*.[5]

Mucin. The nubecula consists, as shown by K. MÖRNER,[6] of a mucoid which contains 12.74 per cent N and 2.3 per cent S. This mucoid, which apparently originates in the urinary passages, may pass to a slight extent into solution in the urine. In regard to the nature of the mucins and nucleoalbumins otherwise occurring in the urine we refer the reader to the pathological constituents of the urine.

Ptomaines and *leucomaines*, or poisonous substances of an unknown kind, which are often described as alkaloidal substances, occur in normal urine, as shown by earlier investigations (POUCHET, BOUCHARD, ADUCCO and others [7]) and also by recent researches of KUTSCHER, LOHMANN and ENGELAND. The *trimethyl-*

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[1] Deutsch. med. Wochenschr., 1905; see also Signorelli, Bioch. Zeitschr., **39**.

[2] See Jastrowitz, Arch. f. exp. Path. u. Pharm., **59**; Walker Hall, Bioch. Journ., **1**; Brugsch and Schittenhelm, Zeitschr. f. exp. Path. u. Therap., **4**.

[3] Pons, Hofmeister's Beiträge, **9**; Sasaki, *ibid.*, **9**; Savarè, *ibid.*, **9** and **11**; Ebbecke, Bioch. Zeitschr., **13**.

[4] Rockwood, Arch. f. (Anat. u.) Physiol., 1895; Oertel, Zeitschr. f. physiol. Chem., **26**, which cites the other works. See also Keller, Zeitschr. f. physiol. Chem., **29**; Mandel and Oertel, N. Y. Univ. Bull. Med. Sciences, **1**; and Maly's Jahresber., **31**; Symmers, Journ. of Path. and Bact., **10**.

[5] In regard to the literature on enzymes in the urine, see Huppert-Neubauer, 599. In regard to trypsin in the urine see Johansson, Zeitschr. f. physiol. Chem., **85**.

[6] Skand. Arch. f. Physiol., **6**.

[7] A complete bibliography on the ptomaines and leucomaines of the urine is found in Huppert-Neubauer, 403.

amine, which originates from the phosphatides, and was first detected by DE FILIPPI and later by K. BAUER, belong to the leucomaines and also the bases found by KUTSCHER and by KUTSCHER and LOHMANN, namely, *methyl guanidine* (also found by ACHELIS), *dimethylguanidine*, *novain* (previously found by DOMBROWSKI), *reductonovain*, $C_7H_{17}NO_2$, *gynesin*, $C_{19}H_{22}N_2O_2$ (from female urine) *mingin*, $C_{12}H_{18}N_2O_2$, *vitiatin* (Chapter X) and *methylpyridine chloride*, which is not a leucomaine, but is probably derived from smoking tobacco or from drinking coffee. The imidazole derivatives *histidine* and *imidazolamino-acetic acid* found by KUTSCHER and ENGELAND also belong to this group and the *urohypertensin* and *urohypotensin* of ABELOUS and BARDIER.[1]

Under pathological conditions the quantity of leucomaines and other bodies may be increased (BOUCHARD, LÉPINE and GEURIN, VILLIERS, GRIFFITHS, ALBU, and others). Within the last few years the poisonous properties of urine have been the subject of more thorough investigation, especially by BOUCHARD. He found that the night urine is less poisonous than the day urine, and that the poisonous constituents of the day and night urine have not the same action. In order to be able to compare the toxic power of the urine under different conditions, BOUCHARD determines the UROTOXIC COEFFICIENT, which is the weight of rabbit in kilos that is killed by the quantity of urine excreted in twenty-four hours by 1 kilo of the person experimented upon.[2]

Many substances have been observed in animal urine which are not found in human urine. To these belong the above-described *kynurenic acid*, *urocanic acid*, which according to HUNTER is imidazolacrylic acid, also found in dog's urine; *damaluric acid* and *damolic acid* (according to SCHOTTEN,[3] probably a mixture of benzoic acid with volatile fatty acids), obtained by the distillation of cow's urine; and lastly *lithuric acid*, found in the urinary concrements of certain animals.

III. INORGANIC CONSTITUENTS OF URINE.

Chlorides. The chlorine occurring in the urine is undoubtedly combined with the bases contained in this excretion; the chief part is in combination with sodium. In accordance with this, the quantity of chlorine in the urine is generally expressed as NaCl.

The question as to whether a part of the chlorine contained in the urine exists as organic combinations, as considered by BERLIOZ and LEPINOIS, is still disputed, although recently BAUMGARTEN[4] has supported this view.

The quantity of chlorine combinations in the urine is subject to considerable variation. In general the amount from a healthy adult on a

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[1] de Filippi, Zeitschr. f. physiol. Chem., **49**; Bauer, Hofmeister's Beiträge, **11**; Kutscher, Zeitschr. f. physiol. Chem., **51**, with Lohmann, *ibid.*, **48** and **49**; Achelis, *ibid.*, **50**; Engeland, *ibid.*, **57**, and Münch. med. Wochenschr., **55**; Abelous and Bardier. Maly's Jahresb., **39** and **40**.

[2] See footnote 7, page 757.

[3] Hunter, Journ. of biol. Chem., **11**; Schotten, Zeitschr. f. physiol. Chem., **7**.

[4] Berlioz and Lepinois, see Chem. Centralbl., 1894, **1**, and 1895, **1**; also Petit and Terrat, *ibid.*, 1894, **2**, and Vitali, *ibid.*, 1897, **2**; Ville and Moitessier, Maly's Jahresber., **31**; Meillère, *ibid.*; Bruno, *ibid.*, 452; Baumgarten, Zeitschr. f. exp. Path. u. Therap., **5**.

mixed diet is 10–15 grams of NaCl per twenty-four hours. The quantity of common salt in the urine depends chiefly upon the amount of salt in the food, with which the elimination of chlorine increases and decreases. The free drinking of water also increases the elimination of chlorine, which is greater during activity than during rest (at night). Certain organic chlorine combinations, such as chloroform, may increase the elimination of inorganic chlorides by the urine (ZELLER, KAST [1]).

In diarrhœa, in quick formation of large transudates and exudates, also in specially marked cases of acute febrile diseases at the time of the crisis, the elimination of NaCl is materially decreased. The excretion of chlorine may vary considerably in disease, but still the NaCl taken with the food has here, as in physiological conditions, a great influence on the NaCl excretion.[2]

The *quantitative estimation of chlorine* in the urine is most simply performed by titration with silver-nitrate solution. The urine must not contain either proteid (which if present must be removed by coagulation) or iodine or bromine compounds.

In the presence of bromides or iodides evaporate a measured quantity of the urine to dryness, fuse the residue with saltpeter and soda, dissolve the fused mass in water, and remove the iodine or bromine by the addition of dilute sulphuric acid and some nitrite, and thoroughly shake with carbon disulphide. The liquid thus obtained may now be titrated with silver nitrate according to VOLHARD'S method. The quantity of bromide or iodide is calculated as the difference between the quantity of silver-nitrate solution used for the titration of the solution of the fused mass and the quantity used for the coresponding volume of the original urine.

The otherwise excellent titration method of MOHR, according to which we titrate with silver nitrate in neutral liquids, using neutral potassium chromate as an indicator, cannot be used directly on the urine in careful work. Organic urinary constituents are also precipitated by the silver salt, and the results are therefore somewhat high for the chlorine. If this method is to be employed, the organic urinary constituents must be destroyed, by incineration after the addition of saltpeter free from chlorine.

According to BANG and LARSSON [3] the disturbing substances which react with $AgNO_3$ can be removed by shaking with blood-charcoal. The value of this suggestion is essentially diminished, because every blood-charcoal cannot be used, and therefore a special testing of the blood-charcoal must be done.

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[1] Zeller, Zeitschr. f. physiol. Chem., 8; Kast, *ibid.*, 11; Vitali, Chem. Centralbl., 1899, 2.

[2] On the elimination of chlorine in disease, see Albu and Neuberg, Physiol. u. Pathol. des Mineralstoffwechsels, Berlin, 1906.

[3] Bioch. Zeitschr., 49.

The silver-nitrate solution may be a N/10 one. It is often made of such a strength that each cubic centimeter corresponds to 0.006 gram Cl or 0.01 gram NaCl. This last-mentioned solution contains 29.075 grams of $AgNO_3$ in 1 liter.

FREUND and TOEPFER, as well as BÖDTKER,[1] have suggested modifications of MOHR'S method.

VOLHARD'S METHOD. Instead of the preceding determination, VOLHARD'S method, which can be performed directly on the urine, may be employed. The principle is as follows: All the chlorine from the urine acidified with nitric acid is precipitated by an excess of silver nitrate, filtered, and in a measured part of the filtrate the quantity of silver added in excess is determined by means of a sulphocyanide solution. This excess of silver is completely precipitated by the sulphocyanide, and a solution of some ferric salt, which, as is well known, gives a blood-red reaction with the smallest quantity of sulphocyanide, is used as an indicator.

We require the following solutions for this titration: 1. A silver-nitrate solution which contains 29.075 grams of $AgNO_3$ per liter, and of which each cubic centimeter corresponds to 0.01 gram NaCl or 0.00607 gram Cl. 2. A saturated solution at the ordinary temperature of chlorine-free iron alum or ferric sulphate. 3. Chlorine-free nitric acid of a specific gravity of 1.2. 4. A potassium-sulphocyanide solution which contains 8.3 grams KCNS per liter, and of which 2 cc. corresponds to 1 cc. of the silver-nitrate solution.

About 9 grams of potassium sulphocyanide are dissolved in water and diluted to 1 liter. The quantity of KCNS contained in this solution is determined by the silver-nitrate solution in the following way: Measure exactly 10 cc. of the silver solution and treat it with 5 cc. of nitric acid and 1–2 cc. of the ferric-salt solution and dilute with water to about 100 cc. Now the sulphocyanide solution is added from a burette, constantly stirring until a permanent faint-red coloration of the liquid takes place. The quantity of sulphocyanide found in the solution by this means indicates how much it must be diluted to be of the proper strength. Titrate once more with 10 cc. of $AgNO_3$ solution and correct the sulphocyanide solution by the careful addition of water until 20 cc. exactly corresponds to 10 cc. of the silver solution.

The determination of the chlorine in the urine is performed by this method in the following way: Exactly 10 cc. of the urine are placed in a flask which has a mark corresponding to 100 cc. and which is provided with a stopper; 5 cc. of nitric acid are added; dilute with about 50 cc. of water and then allow exactly 20 cc. of the silver-nitrate solution to flow in. Close the flask with the stopper and shake well, remove the stopper and wash it with distilled water into the flask, and fill the flask to the 100-cc. mark with distilled water. Close again with the stopper, carefully mix by shaking, and filter through a dry filter. Measure off 50 cc. of the filtrate by means of a dry pipette, add 3 cc. of ferric-salt solution, and allow the sulphocyanide solution to flow in until the liquid above the precipitate has a permanent red color. The calculation is very simple. For example, if 4.6 cc. of the sulphocyanide solution

[1] Freund and Toepfer, see Maly's Jahresber., 22; Bödtker, Zeitschr. f. physiol. Chem., 20.

was necessary to produce the final reaction, then for 100 cc. of the filtrate (=10 cc. urine) 9.2 cc. of this solution are necessary. 9.2 cc. of the sulphocyanide solution corresponds to 4.6 cc. of the silver solution, and since 20−4.6=15.4 cc. of the silver solution was necessary to completely precipitate the chlorine in 10 cc. of the urine, then 10 cc. contains 0.154 gram of NaCl. The quantity of sodium chloride in the urine is therefore 1.54 per cent, or 15.4 p. m. If we always use 10 cc. for the determination, and always 20 cc. of $AgNO_3$ solution, and dilute with water to 100 cc., the quantity of NaCl in 1000 parts of the urine is found by subtracting from 20 the number of cubic centimeters of sulphocyanide (R) required with 50 cc. of the filtrate. The quantity of NaCl p. m. therefore under these circumstances=20−R, and the percentage of $NaCl=\frac{20-R}{10}$.

If it is necessary to destroy the organic urinary constituents before titration, this can be best performed, according to DEHN,[1] by evaporating the urine (10 cc.) to dryness on the water-bath after the addition of a small amount of sodium peroxide, then faintly acidifying with nitric acid and then titrating according to VOLHARD. Incineration is unnecessary.

For the approximate estimation of chlorine in the urine EKEHORN has made use of VOLHARD'S titration method by using for the determination a glass tube closed at one end and divided into half cubic centimeters and called the chlorometer. The reagents necessary are: (*a*) a mixture of 20 cc. silver-nitrate solution (according to VOLHARD), 5 cc. nitric acid and water to 100 cc.; (*b*) 40 cc. sulphocyanide solution and 60 cc. of a ferric alum, chlorine free and saturated at the ordinary temperature. The silver-nitrate solution, of which each cubic centimeter corresponds to 0.002 gm. NaCl, is equivalent to the iron sulphocyanide solution. First 2 cc. of the urine are placed in the graduated tube and then 0.5 cc. sulphocyanide solution, and the silver-nitrate solution gradually added (shaking the tube closed with a rubber stopper) until the coloration of the sulphocyanide just disappears. 0.5 cc. is subtracted from the silver solution for the 0.5 cc. of the sulphocyanide; the tube is so graduated that the quantity of NaCl in the urine in parts per thousand is read off directly on the tube. The difference between these results and those obtained by VOLHARD'S titration method amounts only, according to C. TH. MÖRNER,[2] to 0.25 to at most 0.5 p. m.

The approximate estimation of chlorine in the urine (which must be free from protein) is made by strongly acidifying with nitric acid and then adding to it, drop by drop, a concentrated silver-nitrate solution (1:8). In a normal quantity of chlorides the drop sinks to the bottom as a rather compact cheesy lump. In diminished quantity of chlorides the precipitate is less compact and coherent, and in the presence of very little chlorine a fine white precipitate or only a cloudiness or opalescence is obtained.

Phosphates. Phosphoric acid occurs in acid urines partly as dihydrogen, MH_2PO_4, and partly as monohydrogen, M_2HPO_4, phosphates, both of which may be found in acid urines at the same time. The proportion of these may vary considerably; acid urines contain chiefly dihydrogen phosphate and in many cases the urine seems to contain only dihydrogen phosphate and sometimes indeed only a small quantity of phos-

[1] Zeitschr. f. physiol. Chem., **44**.

[2] Ekehorn, Hygiea, Stockholm, 1906; Mörner, Upsala Läkaref. Förh. (N. F.), **11**.

phoric acid. The total quantity of phosphoric acid varies and depends on the character and the quantity of food. The average quantity of P_2O_5 is in round numbers 2.5 grams, with a variation of 1–5 grams per day. A small part of the phosphoric acid of the urine originates from the burning of organic compounds, such as nuclein, and phosphatides within the organism; on exclusive feeding with substances rich in nuclein or pseudonuclein the quantity of phosphates is essentially increased; still it is undecided to what extent the excretion of phosphoric acid is a measure of the absorption and decomposition of these bodies.[1] The greater part originates from the phosphates of the food, and the quantity of phosphoric acid eliminated is greater when the food is rich in alkali phosphates in proportion to the quantity of lime and magnesium phosphates. If the food contains much lime and magnesia, large quantities of earthy phosphates are eliminated by the excrement; and even though the food contains considerable amounts of phosphoric acid in these cases, the quantity excreted by the urine is small. This is especially true of herbivora, in which the kidneys are the chief organs for the excretion of alkali phosphates. In man, according to EHRSTRÖM, the content of lime in the food seems to play no important rôle, as in his experiments about one-half of the phosphoric acid taken as $CaHPO_4$ was absorbed, still the extent of phosphoric-acid excretion through the urine depends in man not only upon the total quantity of phosphoric acid in the food, but also upon the relative amounts of the alkaline earths and the alkali salts of the food. In carnivora, in which phosphate injected subcutaneously is eliminated by the intestine (BERGMANN), the urine is habitually poor in phosphates.[2]

As the extent of the elimination of phosphoric acid is mostly dependent upon the character of the food and the absorption of the phosphates in the intestine, it is apparent that the relation between the nitrogen and phosphoric-acid excretion cannot run parallel. This is in fact so, and, according to EHRSTRÖM, the organism has the power of accumulating large quantities of phosphorus for a relatively long time independent of the condition of the nitrogen balance. With a certain regular food the relation between nitrogen and phosphoric acid in the urine can be kept almost constant. Thus on feeding with an exclusive meat diet, as observed by VOIT[3] in dogs, when the nitrogen and phosphoric acid

[1] See A. Gumlich, Zeitschr. f. physiol. Chem., 18; Roos, *ibid.*, 21; Weintraud, Arch. f. (Anat. u.) Physiol., 1895; Milroy and Malcolm, Journ. of Physiol., 23; Röhmann and Steinitz, Pflüger's Arch., 72; Loewi, Arch. f. exp. Path. u. Pharm., 44 and 45.

[2] Ehrström, Skand. Arch. f. Physiol., 14; Bergmann, Arch. f. exp. Path. u. Pharm., 47.

[3] Physiologie des allgemeinen Stoffwechsels und der Ernährung in L. Hermann's Handbuch, 6, Thul. 1, 79.

(P_2O_5) of the food exactly reappeared in the urine and feces, the relation was 8.1:1. In starvation, as shown by the compilation of R. TIGERSTEDT,[1] the phosphorized constituents of the body are destroyed to a much greater extent than when food very poor in phosphorus is given. In starvation this relation is changed, namely, relatively more phosphoric acid is eliminated, which seems to indicate that besides flesh and related tissues another tissue rich in phosphorus is largely destroyed. The starvation experiments show that this is the bone-tissue. According to PREYSZ, OLSAVSZKY, KLUG, I. MUNK and MAILLARD[2] the elimination of phosphoric acid is considerably increased by intense muscular work.

As the phosphoric acid is in part derived from the nucleins, it would be expected that in those diseases in which the excretion of purine bodies was increased the phosphoric acid would also be augmented. This is not the case, and indeed we have observed cases with an increased elimination of purine bodies with a diminution in the phosphoric-acid excretion. Cases of leucæmia have been observed in which the phosphoric-acid excretion was reduced, although there was a pronounced increase in the number of leucocytes. In these cases there may be a subsequent excretion or a retention of phosphoric acid. This last condition also occurs in inflammatory and renal diseases. The earthy phosphates of the urine sometimes have the tendency of precipitating either spontaneously or after warming, and this has been called *phosphaturia*. We are here dealing with a diminished acidity and, it seems, with a diminished excretion of phosphoric acid and an increased elimination of lime, or at least an essentially different relation between the phosphoric acid and the alkaline earths of the urine, as compared with the normal (PANEK IWANOFF, SOETBER and KRIEGER[3]).

Quantitative Estimation of the Total Phosphoric Acid in the Urine. This estimation is most simply performed by titrating with a solution of uranium acetate. The principle of the titration is as follows: A warm solution of phosphates containing free acetic acid gives a whitish-yellow precipitate of uranium phosphate with a solution of a uranium salt. This precipitate is insoluble in acetic acid, but dissolves in mineral acids, and on this account there is always added, in titrating, a certain quantity of sodium-acetate solution. Potassium ferrocyanide is used as the indicator, which does not act on the uranium-phosphate precipitate, but gives a reddish-brown precipitate or coloration in the presence of the

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[1] Skand. Arch. f. Physiol., **16**.

[2] Preysz, see Maly's Jahresber., **21**; Olsavszky and Klug, Pflüger's Arch., **54**; Munk, Arch. f. (Anat. u.) Physiol., 1895; Maillard, Journ. de Physiol. et de Path. **10** and **11**.

[3] Panek, see Maly's Jahresber., **30**, 112; Iwanoff, Biochem. Centralbl., **1**, 710; Soetber and Krieger, Deutsch. Arch. f. klin. Med., **72**; Campani, Biochem. Centralbl., **3**, 616; Tobler, Arch. f. exp. Path. u. Pharm., **52**.

smallest amount of soluble uranium salt. The solutions necessary for the titration are: 1. A solution of a uranium salt of which each cubic centimeter corresponds to 0.005 gram P_2O_5 and which contains 20.3 grams of uranium oxide per liter. 20 cc. of this solution corresponds to 0.100 gram P_2O_5. 2. A solution of sodium acetate. 3. A freshly prepared solution of potassium ferrocyanide.

The uranium solution is prepared from uranium nitrate or acetate. Dissolve about 35 grams uranium acetate in water, add some acetic acid to facilitate solution, and dilute to 1 liter. The strength of this solution is determined by titrating with a solution of sodium phosphate of known strength (10.085 grams crystallized salt in 1 liter, which corresponds to 0.010 gram P_2O_5 in 50 cc.). Proceed in the same way as in the titration of the urine (see below), and correct the solution by diluting with water, and titrate again until 20 cc. of the uranium solution corresponds exactly to 50 cc. of the above phosphate solution.

The sodium-acetate solution should contain 10 grams sodium acetate and 10 grams conc. acetic acid in 100 cc. For each titration 5 cc. of this solution is used with 50 cc. of the urine.

In performing the titration, mix 50 cc. of filtered urine in a beaker with 5 cc. of the sodium acetate, cover the beaker with a watch-glass, and warm over the water-bath. Then allow the uranium solution to flow in from a burette, and when the precipitate does not seem to increase, place a drop of the mixture on a porcelain plate with a drop of the potassium-ferrocyanide solution. If the amount of uranium solution added has not been sufficient, the color will remain pale yellow and more uranium solution must be added; but as soon as the slightest excess of uranium solution has been used the color becomes a faint reddish brown. When this point has been obtained, warm the solution again and add another drop. If the color remains of the same intensity, the titration is ended; but if the color varies, add more uranium solution, drop by drop, until a permanent coloration is obtained after warming, and now repeat the test with another 50 cc. of the urine. The calculation is so simple that it is unnecessary to give an example.

In the above manner one determines the total quantity of phosphoric acid in the urine. If we wish to know the phosphoric acid combined with alkaline earths and with alkalies, we first determine the total phosphoric acid in a portion of the urine and then remove the earthy phosphates in another portion by ammonia. The precipitate is collected on a filter, washed, transferred into a beaker with water, treated with acetic acid, and dissolved by warming. This solution is now diluted to 50 cc. with water, and 5 cc. sodium-acetate solution added, then titrated with uranium solution. The difference between the two determinations gives the quantity of phosphoric acid combined with the alkalies. The results obtained are not quite accurate, as a partial transformation of the monophosphates of the alkaline earths and also calcium diphosphate into triphosphates of the alkaline earths and ammonium phosphate takes place on precipitating with ammonia, and the method gives too high results for the phosphoric acid combined with alkalies and remaining in solution.

Sulphates. The sulphuric acid of the urine originates only to a very small extent from the sulphates of the food. A disproportionately

greater part is formed by the burning within the body of the proteins which contain sulphur, and it is chiefly this formation of sulphuric acid from the proteins which gives rise to the previously mentioned excess of acids over the bases in the urine. The quantity of sulphuric acid eliminated by the urine amounts to about 2.5 grams H_2SO_4 per day. As the sulphuric acid chiefly originates from the proteins, it follows that the elimination of sulphuric acid and the elimination of nitrogen runs almost parallel, and the relation $N:H_2SO_4$ is about 5:1. A complete parallelism can hardly be expected, as in the first place a part of the sulphur is always eliminated as neutral sulphur, and secondly because the small proportion of sulphur in different protein bodies undergoes greater variation as compared with the large proportion of nitrogen contained therein. In general the elimination of nitrogen and sulphuric acid under normal and under diseased conditions seems to run parallel. Sulphuric acid occurs in the urine partly preformed (sulphate-sulphuric acid) and partly as ethereal-sulphuric acid. The first is designated as *A*- and the other as *B*-sulphuric acid.

The *quantity of total sulphuric acid* is determined in the following way, but at the same time the precautions described in other works must be observed. 100 cc. of filtered urine is treated with 5 cc. of concentrated hydrochloric acid and boiled for fifteen minutes. While boiling precipitate with 2 cc. of a saturated $BaCl_2$ solution, and warm for a little while until the barium sulphate has completely settled. The precipitate must then be washed with water and also with alcohol and ether (to remove resinous substances), and then treated according to the usual method.

The separate determination of the sulphate-sulphuric acid and the ethereal-sulphuric acid may be accomplished, according to BAUMANN'S method, by first precipitating the sulphate-sulphuric acid by $BaCl_2$ from the urine acidified with acetic acid, then decomposing the ethereal-sulphuric acid by boiling after the addition of hydrochloric acid, and finally determining the sulphuric acid set free as barium sulphate. A still better method is the following, suggested by SALKOWSKI [1]:

200 cc. of urine are precipitated by an equal volume of a barium solution, which consists of 2 vols. barium hydrate and 1 vol. barium chloride solution, both saturated at the ordinary temperature. Filter through a dry filter, measure off 100 cc. of the filtrate which contains only the ethereal-sulphuric acid, treat with 10 cc. of hydrochloric acid of a specific gravity 1.12, boil for fifteen minutes, and then warm on the water-bath until the precipitate has completely settled and the supernatant liquid is entirely clear. Filter and wash with warm water and with alcohol and ether, and proceed according to the generally prescribed method. The difference between the ethereal-sulphuric acid found and the total quantity of sulphuric acid as determined in a special portion of urine is taken to be the quantity of sulphate-sulphuric acid.

[1] Baumann, Zeitschr. f. physiol. Chem., 1; Salkowski, Virchow's Arch., 79.

FOLIN [1] has suggested a method for estimating the sulphate-sulphuric acid as well as the ethereal-sulphuric acid, and also the total sulphur, which is somewhat different from the ordinary methods.

Nitrates occur in small quantities in human urine (SCHÖNBEIN), and they probably originate from the drinking-water and the food. According to WEYL and CITRON,[2] the quantity of nitrates is smallest with a meat diet and greatest with vegetable food. The average amount is about 42.5 milligrams per liter.

Potassium and Sodium. The quantity of these bodies eliminated by the urine by a healthy adult on a mixed diet is, according to SALKOWSKI,[3] 3–4 grams K_2O and 5–8 grams Na_2O, with an average of about 2–3 grams K_2O and 4–6 grams Na_2O. The proportion of K to Na is ordinarily 3:5. The quantity depends above all upon the food. In starvation the urine may become richer in potassium than in sodium, which results from the lack of common salt and the destruction of tissue rich in potassium. The quantity of potassium may be relatively increased during fever, while after the crisis the reverse is the case.

The quantitative estimation of these bodies is made by the gravimetric methods as described in works on quantitative analysis. In the determination of the total alkalies new methods have been devised by PRIBRAM and GREGOR, and for the potassium alone a method by AUTENRIETH and BERNHEIM.[4]

Ammonia. Some ammonia is habitually found in human urine and in that of carnivora. The quantity in human urine on a mixed diet is an average of 0.7 gram, according to NEUBAUER. MAILLARD [5] found higher values for soldiers, namely 1.11 grams. The ammonia nitrogen relative to the total nitrogen is, on a mixed diet, 3.6–5.8 per cent.

As above stated (page 685), on the formation of urea from ammonia, this quantity may represent the small amount of ammonia which is excluded from the synthesis to urea by being combined with acids formed in excess by combustion and not united with the fixed alkalies. This view is confirmed by the observation that the elimination of ammonia was smaller on a vegetable diet and larger on a rich meat diet than on a mixed diet. After abundant meat feeding BOUCHEZ found, for example, 1.35–1.67 gram NH_3 in twenty-four hours. The relationship of the ammonia elimination to the acid formation in the animal body corresponds also to the unquestioned relation between the hydrochloric acid content of the

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[1] Journ. of Biol. Chem., **1**, and Amer. Journ. of Physiol., **13**.

[2] Schönbein, Journ. f. prakt. Chem., **92**; Weyl, Virchow's Arch., **96**, with Citron, *ibid.*, **101**.

[3] *Ibid.*, **53**.

[4] Pribram and Gregor, Zeitschr. f. analyt. Chem., **38**; Autenreith and Bernheim, Zeitschr. f. physiol. Chem., **37**.

[5] Journ. de Physiol. et de Path., **10**.

gastric juice and the ammonia elimination. Thus SCHITTENHELM found that with a rise in the hydrochloric acid content the percentage of ammonia in the urine was raised and also the reverse. A. LOEB and GAMMELTOFT [1] have also observed a fall in the ammonia elimination a few hours after a meal, although no satisfactory explanation of this behavior has been given. That ammonia plays the rôle of a neutralization medium for the acids produced in the body or introduced therein has been shown by various observations.

In man and certain animals the elimination of ammonia is increased by the introduction of mineral acids; and, as shown by JOLIN,[2] organic acids, such as benzoic acid, which are not destroyed in the body act in a similar manner. The ammonia set free in the protein destruction is in part used in the neutralization of the acids introduced, and in this way a destructive removal of fixed alkalies is prevented.

Acids formed in the destruction of proteins in the body act on the elimination of ammonia like those introduced from without. For this reason the quantity of ammonia in human urine is increased under such conditions and in such diseases where an increased formation of acid takes place, because of an increased metabolism of proteins. This is the case with a lack of oxygen in fevers and diabetes. In the last-mentioned disease, organic acids—β-oxybutyric acid and acetoacetic acid—are produced, which pass into the urine combined with ammonia.[3]

The liver forms urea from the ammonia supplied to it by the blood and it would therefore be expected that in certain diseases of the liver or with insufficient liver function that a diminished urea formation and an increased ammonia elimination should take place. This condition has already been mentioned above (page 685), and as there remarked we must consider whether the abnormal production of acid with increased elimination of neutralization ammonia is primary or whether it is a diminished synthetic activity of the liver.

In close relation to what has been said stands the question whether all of the ammonia occurring in the urine under normal conditions is to be considered as neutralization ammonia. If this were so then probably by introducing large amounts of alkali it would be possible to cause the disappearance of ammonia from the urine. In STADELMANN and BECK-

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[1] Bouchez, Journ. de Physiol. et de Path., **14**; Schittenhelm, Deutsch. Archiv. f. klin. Med., **77**; Adam Loeb, Zeitschr. f. klin. Med., **56**, and Zeitschr. f. Biol., **55**; Gammeltoft, Zeitschr. f. physiol. Chem., **75**.

[2] Jolin, Skand. Arch. f. Physiol., **1**. In regard to the behavior of ammonium salts in the animal body, see Rumpf and Kleine, Zeitschr. f. Biologie, **34**; Kowalewski and Markewicz, Bioch. Zeitschr., **4**, and the works cited on pages 682, 683.

[3] On the elimination of ammonia in disease, see the works of Rumpf, Virchow's Arch., **148**; Hallervorden, *ibid.*

MANN'S experiments this was not possible, still in recent experiments of JANNEY [1] it was possible, by introducing large quantities of sodium citrate, which was burned in the body into carbonate, to reduce the ammonia elimination to very insignificant quantities.

The detection and quantitative estimation of ammonia used to be performed according to the method suggested by SCHLÖSING. The principle of this method is that the ammonia from a measured amount of urine is set free by lime-water in a closed vessel and absorbed by a measured amount of N/10 sulphuric acid. After the absorption of the ammonia the quantity is determined by titrating the remaining free sulphuric acid with a N/10 caustic-alkali solution. This method gives low results, and in exact work we must proceed as suggested by BOHLAND.[2]

The recent methods for estimating the ammonia are all based upon the distillation of the ammonia, after the addition of lime, magnesia, or alkali carbonate, at low temperatures either by the aid of vacuum (NENCKI and ZALESKI, WURSTER, KRÜGER, REICH and SCHITTENHELM and SCHAFFER) or by the aid of a current of air (FOLIN) and then collecting it in a standard acid.

According to the methods suggested by KRÜGER, REICH and SCHITTENHELM [3] 25 cc. of the urine are placed in a distillation-flask with about 10 grams of NaCl and 1 gram of Na_2CO_3, and this distilled at 43° C. and a pressure of 30–40 millimeters Hg with the aid of an air-pump. Alcohol is added to prevent foaming. The ammonia is absorbed in N/10 acid contained in a PELIGOT tube surrounded by ice-water, and when the distillation is finished the acid is retitrated, making use of rosolic acid as indicator. In regard to details, see the original publications. Instead of alkali carbonate a one-half normal solution of barium hydrate in methyl alcohol can be used. According to FOLIN'S [4] method, 25–50 cc. of the urine are treated in a wash-bottle with 1–2 grams soda and 8–10 grams sodium chloride and some petroleum, in order to prevent frothing, and then a current of air is passed through and this passed through a second wash-bottle containing N/10 acid. It has also been suggested (RONCHÈSE, MALFATTI and others) to determine the ammonia by the formol titration. This method is based upon the fact that an ammonium salt yields hexamethylentetramine and free acid with formaldehyde according to the equation $4NH_4Cl+6HCOH=C_6H_{12}N_4+6H_2O+4HCl$. This acid is determined by titration after the addition of formol. FOLIN [5] also recently suggested a method for the quantitative colorimetric estimation of ammonia by the use of NESSLER'S reagent.

Calcium and Magnesium occur in the urine chiefly as phosphates. The quantity of earthy phosphates eliminated daily is somewhat more

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[1] N. Janney, Zeitschr. f. physiol. Chem., **76**, which also contains the literature.

[2] Pflüger's Arch., **43**, 32.

[3] Zeitschr. f. physiol. Chem., **39**; Schaffer, Amer. Journ. of Physiol., **8**, which contains the literature. Henriques and Sórensen, Zeitschr. f. physiol. Chem., **64**.

[4] Folin, Zeitschr. f. physiol. Chem., **37**, and Journ. of biol. Chem., 8; Steel, *ibid.*, 8.

[5] Ronchèse, see Maly's Jahresber., **38**, 321; Malfatti, Zeitschr. f. anal. Chem., **47**; H. Björn-Andersen and M. Lauritzen, Zeitschr. f. physiol. Chem., **64**; L. de Jager, *ibid.*, **62**; Folin and Maccallum, Journ. of biol. Chem., **11**.

than 1 gram, and of this amount $\frac{2}{3}$ is magnesium and $\frac{1}{3}$ calcium phosphate. This statement, as found by RENWALL and GROSS, is not correct, or at least is not true in general, as they found more calcium than magnesium in the urine. LONG and GEPHART [1] obtained similar results. In acid urines the mono- as well as the dihydrogen earthy phosphates are found, and the solubility of the first, among which the calcium salt $CaHPO_4$ is especially insoluble, is particularly augmented by the presence in the urine of dihydrogen alkali phosphates and sodium chloride (OTT [2]). The quantity of alkaline earths in the urine depends on the composition of the food. The lime-salts absorbed are in great part excreted again into the intestine, and the quantity of lime-salts in the urine is therefore no measure of their absorption. The introduction of readily soluble lime-salts or the addition of hydrochloric acid to the food may therefore cause an increase in the quantity of lime in the urine, while the reverse takes place on adding alkali phosphate to the food. According to GRANSTRÖM starvation in rabbits or the introduction of food which yields an acid ash and causes an acid urine produces the same effect as the introduction of acid. The observation of DE JAGER [3] is significant, namely, he found that the partaking of $CaSO_4$ and to a higher degree of $MgSO_4$ causes an increase in the urine ammonia and of acid. Nothing is known with certainty in regard to the constant and regular change in the elimination of calcium and magnesium salts in disease, and in these conditions the excretion is chiefly dependent upon the diet and the formation and introduction of acid.[4]

The quantity of calcium and magnesium is determined according to the ordinary well-known methods.

Iron occurs in the urine only in small quantities, and it does not exist as a salt, but as an organic combination of a colloidal nature. The earlier reports in regard to the iron present seem to show that the quantity ranges from 1 to 11 milligrams per liter of urine. HOFFMANN, NEUMANN and MAYER found lower results—an average of 1.09 and 0.983 milligrams and according to recent determinations of WOLTER and REICH [5] the quantity is about 1 milligram. The quantity of *silicic acid* is ordinarily stated to amount to about 0.3 p. m. H. SCHULZ [6] found

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[1] Renwall, Skand. Arch. f. Physiol., **16**; Gross, Biochem. Centralbl., **4**, 189; Long and Gephart, Journ. Amer. Chem. Soc., **34**.

[2] Zeitschr. f. physiol. Chem., **10**.

[3] Granström, Zeitschr. f. physiol. Chem., **58**; de Jager, Bioch. Zeitschr., **38**.

[4] See page 758, Albu and Neuberg, l. c., and E. Zak, Ueber Harn bei Lungenentzündung, Wien. klin. Wochenschr., **21**.

[5] Kunkel, cited from Maly's Jahresber., **11**; Giacosa, *ibid.*, **16**; Kobert, Arbeiten des Pharm. Inst. zu Dorpat, **7**; Magnier, Ber. d. deutsch. chem. Gesellsch., **7**; Gottlieb, Arch. f. exp. Path. u. Pharm., **26**; Jolles, Zeitschr. f. anal. Chem., **36**; Hoffmann, Zeitschr. f. anal. Chem., **40**; Neumann and Mayer, Zeitschr. f. physiol. Chem., **37**; Wolter, Bioch. Zeitschr., **24**; Reich, *ibid.*, **36**.

[6] Pflüger's Arch., **144**.

0.1046 to 0.2594 grams per day on a mixed diet. Traces of *hydrogen peroxide* also occur in the urine.

The *gases* of the urine are carbon dioxide, nitrogen, and traces of oxygen. The quantity of nitrogen is not quite 1 vol. per cent. The carbon dioxide varies considerably. In acid urines it is hardly one-half as great as in neutral or alkaline urines.

IV. THE QUANTITY AND QUANTITATIVE COMPOSITION OF URINE.

The quantity and composition of urine are liable to great variation. The circumstances which under physiological conditions exercise a great influence are the following: the blood-pressure, and the rapidity of the blood-current in the glomeruli. The quantity of urinary constituents, especially water in the blood; and, lastly, the condition of the secretory glandular elements. Above all, the quantity and concentration of the urine depend on the quantity of water which is introduced into the blood or which leaves the body in other ways. The excretion of urine is increased by drinking freely or by reducing the quantity of water otherwise removed; and it is decreased by a diminished ingestion of water or by a greater loss of water in other ways. Ordinarily in man just as much water is eliminated by the kidneys as by the skin, lungs, and intestine together. At lower temperatures and in moist air, since under these conditions the elimination of water by the skin is diminished, the excretion of urine may be considerably increased. Diminished introduction of water or increased elimination of water by other means—as in violent diarrhœa or vomiting, or in profuse perspiration—greatly diminishes the amount of urine excreted. For example, the urine may sink as low as 500–400 cc. per day in intense summer heat, while after copious draughts of water the elimination of 3000 cc. of urine has been observed during the same time. The quantity of urine voided in the course of twenty-four hours varies considerably from day to day, the average being ordinarly calculated as 1500 cc. for healthy adult men and 1200 cc. for women. The minimum elimination occurs during the early morning between 2 and 4 o'clock; the maximum, in the first hours after waking and from 1–2 hours after a meal.

The quantity of solids excreted per day is nearly constant, even though the quantity of urine may vary, and it is quite constant when the manner of living is regular. Therefore the percentage of solids in the urine is naturally in inverse proportion to the quantity of urine. The average amount of solids per twenty-four hours is calculated as 60 grams. The quantity may be calculated with approximate accuracy from the specific gravity if the second and third decimals of this factor be multiplied by HÄSER'S coefficient, 2.33. The product gives the amount of solids in 1000 cc. of urine, and if the quantity of urine eliminated in twenty-four hours be measured, the quantity of solids in twenty-four hours may be easily calculated. For example, 1050 cc. of urine of a specific gravity 1.021 was

eliminated in twenty-four hours; therefore the quantity of solids excreted was $21 \times 2.33 = 48.9$ and $\frac{48.9 \times 1050}{1000} = 51.35$ grams. LONG[1] has made a new determination of the coefficient for the specific gravity taken at 25° C. and finds that it is equal to 2.6, which almost corresponds to HÄSER's coefficient at 15° C.

Those bodies which, under physiological conditions, affect the density of the urine are common salt and urea. The specific gravity of the first is 2.15 and the last only 1.32, so it is easy to understand, when the relative proportion of these two bodies essentially deviates from the normal, why the above calculation from the specific gravity is not exact. The same is true when a urine poor in normal constituents contains large amounts of foreign bodies, such as albumin or sugar.

As above stated, the percentage of solids in the urine generally decreases with a greater elimination, and a very considerable excretion of urine (*polyuria*) has therefore, as a rule, a lower specific gravity. An important exception to this rule is observed in urine containing sugar (*diabetes mellitus*), in which there is a copious excretion with a very high specific gravity due to the sugar. In cases where very little urine is excreted (*oliguria*), e.g., during profuse perspiration, in diarrhœa, and in fevers, the specific gravity of the urine is as a rule very high; the percentage of solids is also high and the urine has a dark color. Sometimes, as for example, in certain cases of albuminuria, the urine may have a low specific gravity notwithstanding the oliguria, and be poor in solids and light in color.

In certain cases it is interesting to know the relation between the carbon and the nitrogen, or the quotient C/N. This factor may vary between 0.6 and 1; as a rule, it amounts on an average to 0.87, but changes according to the nature of the food and is higher after a diet rich in carbohydrates than after food rich in fat (PREGL, TANGL, LANGSTEIN and STEINITZ). According to MAGNUS-ALSLEBEN it rises after body exertion, but in healthy individuals the variation is independent of the kind of food. In the urine analyses of BOUCHEZ[2] a variation between 0.62 and 0.90 was observed which showed no regular relation to the food.

On account of the great variations which the composition of the urine shows it is difficult and of little value to give a tabular review of the composition of the urine. The following table contains only approximate values and it must not be overlooked that the results are not given for 1000 parts of urine, but only approximate figures for the quantities

[1] Journ. Amer. Chem. Soc., 25.

[2] Pregl, Pflüger's Arch., 75, which contains the earlier literature. Tangl, Arch. f. (Anat. u.) Physiol., 1899, Suppl.; Langstein and Steinitz, Centralbl. f. Physiol., 19; Magnus-Alsleben, Zeitschr. f. klin. Med., 68, Bouchez, footnote 1, page 767.

of the most important constituents which are eliminated during the course of twenty-four hours in a volume of 1500 cc. of urine. These figures apply only to a diet which corresponds to Voit's standard figures, namely 118 grams protein, 56 grams fat, and 500 grams carbohydrate per day, and to a man of average weight.

Daily quantity of solids = 55–70 grams.

Organic constituents	35–45 grams.	Inorganic constituents	20–25 grams.
Urea	25–35.0 grams.	Sodium chloride (NaCl)	10–15.0 grams.
Uric acid	0.7 "	Sulphuric acid (H_2SO_4)	2.5 "
Creatinine	1.5 "	Phosphoric acid (P_2O_5)	2.5 "
Hippuric acid	0.7 "	Potash (K_2O)	3.3 "
		Ammonia (NH_3)	0.7 "
		Magnesia (MgO) } Lime (CaO) }	0.8 "

Urine contains on an average 40 p. m. solids. The quantity of urea is about 20 p. m., and common salt about 10 p. m.

The physico-chemical methods are being used in urinary analysis even to a greater extent than in the analysis of other animal fluids. A great number of cryoscopic determinations, but fewer conductivity determinations, have been made. A constant relation between the values found by physico-chemical methods and the analytical methods has been sought, for example, between the freezing-point depression and the specific gravity or the common salt content and others; or have been made to find certain constants in the composition of the urine based upon the results of various methods, and in this way to obtain an explanation as to the mechanism of the excretion of urine in order to apply them for diagnostic purposes. The results obtained are, as is to be expected, so variable and dependent upon so many conditions which cannot be controlled that definite conclusions must be drawn with the greatest caution. In regard to the value and usefulness of the various constants and relations which are based upon theoretical considerations, opinions are unfortunately still too divergent and as the plan and scope of this book do not allow of more detailed description of these facts we must refer to larger works on the urine and diseases of the kidneys.

V. CASUAL URINARY CONSTITUENTS.

The casual appearance, in the urine, of medicinal agents or of urinary constituents resulting from the introduction of foreign substances into the organism is of practical importance, because such compounds may interfere in certain urinary investigations; they also afford a good means of determining whether certain substances have been introduced into the organism or not. From this point of view a few of these bodies will be spoken of in a following section (on the pathological urinary constituents). The presence of these foreign bodies, in the urine, is of special interest in those cases in which they serve to elucidate the chemical transformations which certain substances undergo within the organism. As inorganic substances generally leave the body unchanged,[1] they are of very little

[1] In regard to the behavior of certain of these bodies, see Heffter, Die Ausscheidung körperfremden Substanzen im Harn, Ergebnisse d. Physiol., 2, Abt. 1.

interest from this standpoint; but the changes which certain organic substances undergo when introduced into the animal body may be studied by the transformation products as found in the urine.

The bodies belonging to the **fatty series** undergo, though not without exceptions, a combustion leading toward the final products of metabolism; still, often a greater or smaller part of the bodies in question escape oxidation and appear unchanged in the urine. A part of the acids belonging to this series, which are otherwise decomposed into water and carbonates, and render the urine neutral or alkaline, may act in this manner. The *volatile fatty acids* poor in carbon are less easily oxidized than those rich in carbon, and they therefore pass unchanged into the urine in large amounts. This is especially true of formic and acetic acids (SCHOTTEN, GRÉHANT and QUINQUAUD [1]). In birds, according to GAGLIO and GIUNTI, *oxalic acid* is not oxidized. Opinions on the behavior of oxalic acid in mammalia and man, are conflicting; the investigations of SALKOWSKI and especially of HILDEBRANDT and DAKIN [2] show that oxalic acid, when introduced in medium amounts, is in part oxidized in the animal body. *Racemic acid*, *d-l* tartaric acid, passes (in dogs) in part into the urine, and this unburned part is optically inactive according to NEUBERG and SANEYOSHI. The statement of BIRON [3] that *l*-tartaric acid is more readily burned than *d*-tartaric acid is accordingly incorrect, and the *d-l*-tartaric acid therefore does not belong to those substances which are asymmetrically attacked in the animal body. *Malic acid* and *citric acid* belong to those acids which are in great part burned in the body.[4]

The destruction of normal *fatty acids* with several membered chains takes place, our belief being based upon the work of KNOOP and DAKIN [5] especially, in an oxidation in the β-position, i.e., in the group which is in the β-position to the carboxyl group at the end. The conversion into an

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[1] Schotten, Zeitschr. f. physiol. Chem., 7; Gréhant and Quinquaud, Compt. Rend., 104.

[2] Gaglio, Arch. f. exp. Path. u. Pharm., 22; Giunti, Chem. Centralbl., 1897, 2; Marfori, Maly's Jahresber., 20 and 27; Pohl, Arch. f. exp. Path. u. Pharm., 37; Salkowski, Berl. klin. Wochenschr., 1900; Pierallini, Virchow's Arch., 160; Stradomsky, *ibid.*, 163; Klemperer and Tritschler, Zeitschr. f. klin. Med., 44; Hildebrandt, Zeitschr. f. physiol. Chem., 35; Dakin, Journ. of biol. Chem., 3.

[3] Biron, Zeitschr. f. physiol. Chem., 25; Neuberg and Saneyoshi, Bioch. Zeitschr., 36.

[4] Pohl, Arch. f. exp. Path. u. Pharm., 37, which also contains reports on the intermediary products formed in the oxidation of the fatty bodies; K. Ohta, Bioch. Zeitschr., 44.

[5] F. Knoop, Hofmeister's Beiträge, 6 and Habilit.-Schrift, Freiburg, 1904; Dakin, Journ. of biol. Chem., 4, 5, 6 and 9.

acid having two carbon atoms less takes place according to this assumption according to the formula:

$$R.CH_2.CH_2.COOH \rightarrow R.CH(OH).CH_2.COOH \quad R.CO.CH_2.COO \leftrightarrows H \rightarrow R.COOH.$$

The animal body has therefore the ability to transform oxyacids (alcohol acids) into keto-acids by oxidation as well as the reverse, the conversion of keto-acids into oxyacids, and this behavior, which is indicated by the above formula, makes it difficult to state which products are primary and which are secondary. As example of such a reversible process we will mention the following; the β-oxybutyric acid $CH_3.CH(OH).CH_2COOH$ is transformed by oxidation into the keto-acid, acetoacetic acid, $CH_3.CO.CH_2COOH$, and this latter by reduction is changed into β-oxybutyric acid. Both processes may take place, as FRIEDMANN and MAASE, DAKIN and WAKEMAN [1] have shown, in the liver, and as these two so-called acetone bodies have great importance in diabetes, they may serve also as an example of the first stages of a β-oxidation (of n. butyric acid).

Most of the investigations on the demolition of fatty acids have been carried out by KNOOP, DAKIN, FRIEDMANN and others upon substituted, especially phenyl-substituted fatty acids, and in speaking of the behavior of the cyclic compounds we will discuss the behavior of these.

The *amino-acids* are, when large amounts are introduced into the animal body, eliminated unchanged, and even under physiological conditions traces of the amino-acids formed in the animal body can pass into the excretions—glycocoll in the urine and serine in the perspiration. Otherwise they are as a rule decomposed and a deamidation takes place, the ammonia split off serving for material for the formation of urea. The two components of a racemic α-amino-acid behave differently in that the alien component is burned with greater difficulty and less completely than the component occurring in the body protein, which is burned more readily and more completely.

In the demolition of the α-amino-acids, fatty acids, poorer in carbon, are formed; the detailed manner of this demolition has been explained in various ways.

According to a long-accepted view it was believed that a hydrolytic splitting off of ammonia with the formation of the corresponding oxyacid (alcohol acid) took place, according to the formula $R.CHNH_2.COOH + H_2O = R.CH(OH).COOH + NH_3$, and then a further demolition to

[1] Friedmann and Maase, Bioch. Zeitschr., 27; Dakin and Wakeman, Journ. of biol. Chem., 8.

R.COOH. The appearance of lactic acid in the urine of rabbits after feeding alanine is an example of such deamidation.[1] The possibility is not excluded that in the first place the keto-acid, pyruvic acid, $CH_3.CO.COOH$, is formed from the alanine and then the lactic acid, $CH_3.CHOH.COOH$, formed from this as a secondary reduction product.

In agreement with the views of NEUBAUER[2] it is now rather generally conceded that the hydrolytic deamidation is not as important as the oxidative deamidation, with the formation of keto-acids $R.CH(NH_2).COOH+O=R.CO.COOH+NH_3$, although this is not the only possibility. The proofs for the correctness of this view have been obtained essentially by experiments with aromatic amino-acids and will be given as examples of such deamidation.

DAKIN and DUNDLEY[3] have shown that all α-amino-acids investigated by them can be decomposed under special conditions so that they to a certain degree yield ammonia and an α-keto-aldehyde..

$$R.CH.NH_2.COOH \rightarrow R.CO.CHO+NH_3.$$

Thus, with alanine, and as the reaction to all appearances is reversible, they consider the relationship between alanine and lactic acid is as follows:

$$CH_3.CH.NH_2.COOH \rightleftarrows CH_3.CO.CHO \rightleftarrows CH_3CHOH.COOH.$$

They also found it probable, that the α-keto-aldehydes represent the first step in the demolition of the α-amino-acids whereby the regular demolition of these acids takes place over the α-keto-acids and not over oxyacids, which explains also the formation of sugar from certain amino-acids (over methylglyoxal as intermediary step).

The deamidation after previous oxidation with the formation of keto-acids has awakened special interest because recently in perfusion experiments on dog-livers the reverse process, namely a synthesis of amino-acids from keto-acids (in part also from oxyacids) and ammonia has been performed (KNOOP, EMBDEN and SCHMITZ, KONDO[4]). Among such syntheses we can here call attention to the synthesis in the dog-liver of alanine, phenylalanine and tyrosine from pyruvic acid (also lactic acid), phenylpyruvic acid and *p*-oxyphenyl pyruvic acid, or of α-amino-*n*-butyric acid from α-keto-butyric acid (all as ammonium salts).

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[1] See Langstein and Neuberg, Arch. f. (Anat. u.) Physiol., 1903. Suppl. Bd.

[2] Deutsch. Arch. f. klin. Med., **95**, and Habilit. Schrift., Leipzig, 1908. See also further on in regard to the literature on the demolition of the aromatic amino-acids.

[3] Journ. of biol. Chem., **14**.

[4] Knoop, Zeitschr. f. physiol. Chem., **67** and **71**; Embden and Schmitz, Bioch. Zeitschr., **29** and **38**; Kondo, *ibid.*, **38**.

The residue of the amino-acids remaining after deamidation can naturally, according to the rule governing the fatty acids, be burned and in certain cases this combustion takes place with the formation of *acetone bodies* (which see). The fatty acid residue can also be used, besides in the synthesis of amino-acids, also in the synthesis of other substances, and in Chapter VII the formation of carbohydrates from amino-acids has been mentioned.

Among the amino-acids the *cystine*, or better the *cysteine*,

$$CH_2.(SH).CH(NH_2).COOH,$$

show a special behavior. On oxidation in the SH group and splitting off of CO_2 (see page 149) it is transformed into a new amino-acid, taurine $(H_2N)CH_2.CH_2(SO_2OH)$. Taurine, which when conjugated with cholic acid forms taurocholic acid, occurring in the bile and which is habitually decomposed in the intestine or other parts of the animal body, can when introduced as such into the human body, at least in part, be eliminated in the urine as such or as *tauro-carbamic acid*, $H_2N.CO.NH.C_2H_4.SO_2OH$ (SALKOWSKI [1]). Otherwise as end-products of the demolition of cystine and taurine an increased elimination of urinary sulphur, sulphuric acid and thiosulphate, have been observed (BLUM, ABDERHALDEN and SAMUELY [2]). The sulphydryl group of cysteine also serves in the formation of sulphocyanide, which is formed from the *nitriles*, introduced into the animal body, by the HCN (LANG). The loosely combined sulphur of the proteins, according to the observations of PASCHELES, in alkaline reaction and body temperature, can be readily transformed, with the cyan alkali into sulphocyanide alkali. The alkali sulphocyanides when ingested are almost quantitatively eliminated in the urine, according to POLLAK.[3]

By substituting one of the hydrogen atoms in the NH_2 group of normal α-amino-acids by an alkyl radical (methyl) the combustion of the acids of the series C_2 and C_4 is considerably retarded and almost entirely prevented in the members of the C_5 and C_6 series (FRIEDMANN).[4] *Sarcosine* (methyl glycocoll), $(CH_3)NH.CH_2.COOH$, is not readily burnt, and therefore passes in great part unchanged into the urine, but perhaps also passes in small part into the corresponding uramino-acid, *methylhydantoic acid*, $NH_2.CO.N(CH_3).CH_2.COOH$ (SCHULTZEN [5]), is an example

[1] Ber. d. d. Chem. Gesellsch., 6, and Virchow's Arch., 58.

[2] Blum, Hofmeister's Beiträge, 5; Abderhalden and Samuely, Zeitschr. f. physiol. Chem., 46.

[3] Lang, Arch. f. exp. Path. u. Pharm., 34; Pascheles, *ibid.*; Pollak, Hofmeister's Beiträge, 2.

[4] Hofmeister's Beiträge, 11.

[5] Ber. d. d. Chem. Gesellsch., 5. See also Baumann and v. Mering, *ibid.*, 8, and E. Salkowski, Zeitschr. f. physiol. Chem., 4.

of this kind. Substitution of both hydrogen atoms of the amino-group by methyl groups seems to make the demolition of the amino-acids still more difficult (FRIEDMANN). Ordinary betaine (trimethyl glycocoll) passes, according to KOHLRAUCH,[1] in part unburned into the urine in carnivora as well as herbivora.

The combustion of the aliphatic bodies can be retarded or prevented also by substitutions of other kinds and by combining with other substances.

By *substitution with halogens*, bodies otherwise readily oxidizable are converted into difficultly oxidizable ones. While the aldehydes are readily and completely burnt like the primary and secondary alcohols of the fatty series, the halogen-substituted aldehydes and alcohols, are, on the contrary, difficultly oxidizable. The halogen-substitution products of methane (chloroform, iodoform, and bromoform) are at least in part destroyed, and the corresponding alkali compounds of the halogen pass into the urine.[2]

By *conjugation with sulphuric acid*, the alcohols which are otherwise readily oxidizable may be protected against combustion, and consequently the alkali salt of ethyl-sulphuric acid is not burnt in the body (SALKOWSKI[3]).

Conjugation with other substances can prevent the combustion of the aliphatic bodies as shown in the conjugation of glycocoll with benzoic acid into hippuric acid. A conjugation can also be a mutual protection against the combustion of two bodies as in the case of glucuronic acid and certain substances.

Conjugation with glucuronic acid occurs, according to the investigations of SUNDVIK and especially of O. NEUBAUER, in many substituted as well as non-substitued alcohols, aldehydes, and ketones. *Chloral hydrate*, $CCl_3CH(OH)_2$, passes, after it has been converted into trichlorethyl-alcohol by a reduction, into a levogyrate reducing acid, *urochloralic acid* or trichlorethylglucuronic acid, $CCl_3.CH_2.C_6H_9O_7$ (MUSCULUS and v. MERING). Of the primary alcohols investigated by NEUBAUER[4] (upon rabbits and dogs) methyl alcohol gave no conjugated glucuronic acid, and ethyl alcohol only a small amount. Isobutyl alcohol and active

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[1] Zeitschr. f. Biol., 57.

[2] See Harnack and Gründler, Berlin. klin. Wochenschr., 1883; Zeller, Zeitschr. f. physiol. Chem., 8; Kast, *ibid.*, 11; Binz, Arch. f. exp. Path. u. Pharm., 28; Zeehuisen, Maly's Jahresber., 23.

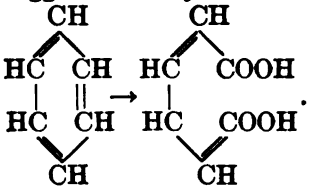
[3] Pflüger's Arch., 4.

[4] Sundvik, Maly's Jahresber., 16; Musculus and v. Mering, Ber. d. deutsch. chem. Gesellsch., 8; also v. Mering, *ibid.*, 15; Zeitschr. f. physiol. Chem., 6; Külz, Pflüger's Arch., 28 and 33; O. Neubauer, Arch. f. exp. Path. u. Pharm., 46; Saneyoshi, Bioch. Zeitschr., 36.

amyl alcohol yielded relatively large quantities. Secondary alcohols produced conjugated glucuronic acids, and indeed to a greater extent than the primary alcohols, especially in rabbits. The ketones are reduced in part into secondary alcohols and are partly excreted as the conjugated acid. This could be shown for acetone with rabbits but not with dogs.

The **homo- and heterocyclic compounds** pass, as far as is known, into the urine as such, or after a previous partial oxidation or synthesis with other bodies, and appear as so-called aromatic compounds. This applies at least to foreign substances that are introduced into the body.

The fact that benzene may be oxidized outside of the body into carbon dioxide, oxalic acid, and volatile fatty acids has been known for a long time; and as in these cases a rupture of the benzene ring must take place, so also, it must be admitted, when aromatic substances undergo a combustion in the animal body, a splitting of the benzene nucleus with the formation of fatty bodies must be the result. If this does not occur, then the benzene nucleus is eliminated with the urine as an aromatic compound of one kind or another. The manner in which this benzene ring is opened is not known. Still JAFFÉ[1] has detected muconic acid in the urine of dogs and rabbits which had been fed for a long time with benzene, and suggest one way in which the benzene can be split in the animal body.



That the demolition of the benzene nucleus occurs in certain cases, as in tyrosine and phenylalanine according to the present view, over homogentisic acid, has already been mentioned. The most striking example of a complete combustion of the benzene nucleus is given by tyrosine, which as previously mentioned (page 737) can be absorbed even in large quantities and decomposed without the observer being able to detect any of the cleavage products of it in the urine. Other examples of readily and at least in greatest part combustible aromatic substances are *phenyl-α-lactic acid*, *p-oxyphenylpyruvic acid* and *α-amino cinnamic acid*. According to JUVALTA and PORCHER *phthalic acid* is also burnt in the animal body. The last investigator found that the three phthalic acids have varying effects, as the *o*-acid is almost completely burnt in dogs, while about 75 per cent of the *m*- and *p*-acids are excreted unconsumed. This corresponds with the rule found by R. COHN,[2] that among the di-derivatives of benzene the ortho-compounds are more

[1] Zeitschr. f. physiol. Chem., **62**.
[2] *Ibid.*, **17**.

readily destroyed than the corresponding meta- and para-compounds. The claims of JUVALTA and PORCHER are unfortunately disputed by PRIBRAM and POHL.[1]

An *oxidation* in the side chain of aromatic compounds is often found, and may also occur in the nucleus itself. As an example, benzene is first oxidized to oxybenzene (SCHULTZEN and NAUNYN), and this is then further in part oxidized into *dioxybenzenes* (BAUMANN and PREUSSE). *Naphthalene* appears to be converted into *oxynaphthalene*, and probably a part also into *dioxynaphthalene* (LESNIK and M. NENCKI). The hydrocarbon with an amino- or imino-group may also be oxidized by a substitution of hydroxyl for hydrogen, especially when the formation of a derivative in the para-position is possible (KLINGENBERG). For example, *aniline*, $C_6H_5.NH_2$, passes into paraminophenol, which latter passes into the urine as its ethereal-sulphuric acid, $H_2N.C_6H_4.O.SO_2.OH$ (F. MÜLLER). *Acetanilid* is in part converted into acetyl paraminophenol (JAFFÉ and HILBERT, K. MÖRNER), and *carbazol* into oxycarbazol (KLINGENBERG).[2]

An *oxidation of the side chain* may occur by the hydrogen atoms being replaced by hydroxyl, or may also take place with the formation of carboxyl; thus, for example, *toluene*, $C_6H_5.CH_3$ (SCHULTZEN and NAUNYN), *ethylbenzene*, $C_6H_5.C_2H_5$, and *propylbenzene*, $C_6H_5.C_3H_7$ (NENCKI and GIACOSA)[3] besides many other bodies, are oxidized into benzoic acid. *Cymene* is oxidized to cumic acid, *xylene* to toluic acid, *methylpyridine* to pyridinecarboxylic acid in the same way.

If *several side chains* are present in the benzene nucleus, then only one is always oxidized into carboxyl. Thus *xylene*, $C_6H_4(CH_3)_2$, is oxidized into *toluic acid*, $C_6H_4(CH_3)COOH$ (SCHULTZEN and NAUNYN); *mesitylene*, $C_6H_3(CH_3)_3$, into *mesitylenic acid*, $C_6H_3(CH_3)_2.COOH$ (L. NENCKI); *cymene*, $(CH_3)_2CH.C_6H_4.CH_3$, into *cumic. acid*, $(CH_3)CH.C_6H_4.COOH$ (M. NENCKI and ZIEGLER[4]).

If the side-chain has several members, then the behavior is different and in these cases the demolition of aromatic amino-acids and fatty acids is especially to be considered.

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[1] Juvalta, Zeitschr. f. physiol. Chem., **13**; Pribram, Arch. f. exp. Path. u. Pharm., **51**; Porcher, Bioch. Zeitschr., **14**; Pohl, *ibid.*, **16**.

[2] Schultzen and Naunyn, Arch. f. (Anat. u.) Physiol., 1867; Baumann and Preusse, Zeitschr. f. physiol. Chem., **3**, 156. See also Nencki and Giacosa, *ibid.*, **4**; Lesnik and Nencki, Arch. f. exp. Path. u. Pharm., **24**; F. Müller, Deutsch. med. Wochenschr., 1887; Jaffé and Hilbert, Zeitschr. f. physiol. Chem., **12**; Mörner, *ibid.*, **13**; Klingenberg, "Studien über die Oxydation aromatischer Substanzen," etc., Inaug.-Diss., Rostock, 1891.

[3] Zeitschr. f. physiol. Chem., **4**.

[4] Nencki, Arch. f. exp. Path. u. Pharm., **1**; Nencki and Ziegler, Ber. d. d. Chem. Gesellsch., **5**; see also O. Jacobsen, *ibid.*, **12**.

The *aromatic amino-acids* are, like the amino-acids in general, decomposed to fatty acids and have one carbon atom less. For example *phenyl-amino-acetic acid* is in part converted into benzoic acid (O. NEUBAUER); *o-* and *m*-tyrosine yield *o-* and *m-oxyphenylacetic acid* respectively (BLUM, FLATOW); *p-chlorphenylalanine* passes according to FRIEDMANN and MAASE into *p-chlorphenylacetic* acid, and *phenyl-α-aminobutyric acid* is changed, as KNOOP[1] showed, into *phenylpropionic acid.* As intermediary steps in this demolition we have, as in the other amino-acids, part the hydrolytic cleavage of NH_2 groups and part the demolition by way of the corresponding keto-acid.

As an example of a demolition of the first kind we have for a long time considered the finding by SCHOTTEN, of mandelic acid

$$C_6H_5.CH(OH).COOH$$

in the urine after feeding *phenylaminoacetic acid,* $C_6H_5.CH(NH_2).COOH$. According to O. NEUBAUER[2] the process is nevertheless of another kind, namely, mandelic acid is produced secondarily by reduction from the intermediarily formed keto-acid, *phenylglyoxylic acid,* $C_6H_5.CO.COOH$. As example of a hydrolytic deamidation we will use the production, as first observed by BLENDERMANN, of *p-oxyphenyl-lactic acid,*

$$HO.C_6H_4.CH_2.CH(OH).COOH$$

from tyrosine (in rabbits). This acid has also been found in the urine by SCHULTZEN and RIESS in acute yellow atrophy of the liver, and by BAUMANN in phosphorus poisoning, although the earlier investigators incorrectly considered the acid as oxymandelic acid. That this acid, which was considered as *oxymandelic acid,* is *l-p*-oxyphenyl-lactic acid has been proved by ELLINGER and KOTAKE and FROMHERZ.[3]

As shown especially by the investigations of O. NEUBAUER the demolition of the aromatic amino-acids passes ordinarily by way of the corresponding *keto-acid.* As stated above (page 737) in regard to the formation of homogentisic acid, the demolition of tyrosine, according to NEUBAUER, passes over the *p-oxyphenylpyruvic acid,* $HO.C_6H_4.CH_2.CO.COOH$. According to him *phenylamino-acetic acid* also yields *phenylglyoxylic acid;*

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[1] Neubauer, Deutsch. Arch. f. klin. Med., **95**; L. Blum, Arch. f. exp. Path. u. Pharm., **59**; Flatow, Zeitschr. f. physiol. Chem., **64**; F. Knoop, *ibid.*, **67**; Friedmann and Maase, Bioch. Zeitschr., **27**.

[2] Schotten, Zeitschr. f. physiol. Chem., 8; O. Neubauer, l. c.

[3] Blendemann, Zeitschr. f. physiol. Chem., **6**; Schultzen and Riess, Chem. Centralbl., 1869; Baumann, Zeitschr. f. physiol. Chem., **6**; Ellinger and Kotake, *ibid.*, **65**; Fromherz, *ibid.*, **70**.

the *m*-tyrosine passes according to FLATOW [1] in part as *m*-oxyphenyl pyruvic acid in the urine. The keto-acids give also the same end products as the corresponding amino-acids. Thus *o*-tyrosine, like *o*-oxyphenyl-pyruvic acid, yields *o*-oxyphenylacetic acid (FLATOW) as end product; the *p-chlorphenylalanine* and the *p-chlorphenylpyruvic acid* pass into the *p*-chlorphenylacetic acid, which is not the case with the oxyacid, the *p-chlorphenyl-lactic acid* (FRIEDMANN and MAASE [2]). This last-mentioned case is an example of the more ready combustibility of the keto-acids as compared to the oxyacids. Another such example is the *p*-oxyphenyl pyruvic acid, which is in great part burned, while the *p*-oxyphenyl-lactic acid is hardly burned at all (KOTAKE, SUWA). A correspondingly different behavior is shown by these two acids in perfusion experiments with the excised liver of the dog. The oxyphenylpyruvic acid, like tyrosine, shows itself to be an acetone former while oxyphenyl-lactic acid, on the contrary, does not (NEUBAUER and GROSS, E. SCHMITZ [3]). The ready combustibility of the keto-acids indicate that these acids and not the oxyacids are the important intermediary cleavage products.

In regard to the demolition of *aromatic fatty acids*, KNOOP [4] has found that the acids with even carbon chains, such as phenyl butyric acid and phenyl caproic acid, are converted into phenylacetic acid, which conjugates with glycocoll to form phenaceturic acid, while the acids with uneven carbon chains, like phenylpropionic and phenylvaleric acid, yield benzoic acid, which then is eliminated as hippuric acid. This behavior is in close agreement with the generally accepted oxidation of fatty at the β-group, for which DAKIN has also given important support. Thus DAKIN found after feeding phenylpropionic acid to cats, that *phenyl-β-oxypropionic acid*, *benzoylacetic acid* and *acetophenone*, the latter passing into benzoic acid or hippuric acid, were formed, which presupposes an oxidation in the β-position. According to the investigations of DAKIN and FRIEDMANN [5] the conditions are still very complicated. Certain of the processes are reversible, oxidations as well as reductions occur, and α-β-unsaturated acids may also be formed as intermediary products. DAKIN as well as FRIEDMANN have obtained cinnamic acid as intermediary product in the demolition of phenylpropionic acid, and

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[1] Neubauer, Deutsch. Arch. f. klin. Med., **95**; Flatow, Zeitschr. f. physiol. Chem., **64**.

[2] Flatow, l. c.; Friedmann and Masse, Bioch. Zeitschr., **27**.

[3] Kotake, Zeitschr. f. physiol. Chem., **69**; Suwa, *ibid.*, **72**; Neubauer and Gross, *ibid.*, **67**; Schmitz, Bioch. Zeitschr., **28**.

[4] Hofmeister's Beiträge, **6**, and Habilit.-Schrift, Freiburg, 1904.

[5] Dakin, Journ. of biol. Chem., **4**, **5**, **6**, **8**, and **9**; Friedmann, see Med. Klinik, No. 28, 1911, and Bioch. Zeitschr., **35**.

this is probably formed from the phenyl-β-oxypropionic acid by the withdrawal of water:

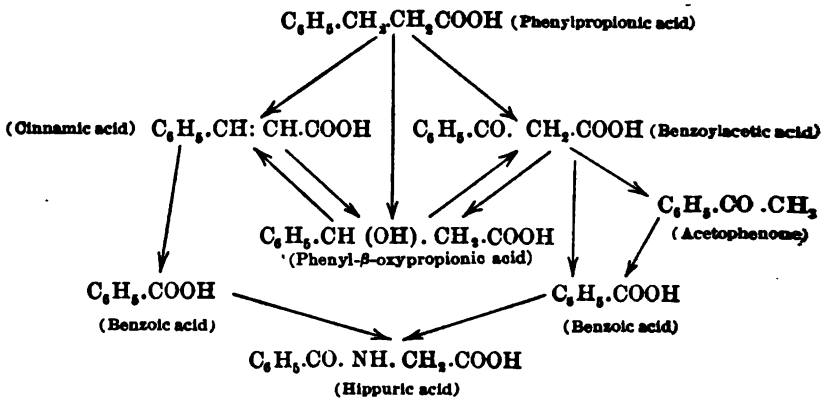
$$C_6H_5.CH(OH).CH_2.COOH - H_2O = C_6H_5.CH:CH.COOH.$$

FRIEDMANN has also (in part with SASAKI)[1] studied the decomposition, of *furfurpropionic acid* and found that pyromucic acid with furfuracrylic acid as intermediary step, was formed:

$$C_4H_3O.CH_2CH_2COOH \rightarrow C_4H_3O.CH:CH.COOH \rightarrow C_4H_3O.COOH.$$

The above-mentioned investigators are therefore of the opinion that the demolition takes place in part over the α-β-unsaturated acids and in part over the β-keto-acids or β-alcohol acids.

According to the investigations of DAKIN and FRIEDMANN and to the schematic illustration which they give, we can consider the demolition of phenylpropionic acid as follows:



Reductions may also occur and besides the examples of the reduction of keto-acids to alcohol-acids, we will mention as further examples the conversion, as observed by E. MEYER,[2] of *nitrobenzene*, $C_6H_5NO_2$, or of *nitrophenol*, $HO.C_6H_4.NO_2$ into aminophenol, $HO.C_6H_4.NH_2$, and also the behavior of *m*-nitro-benzaldehyde in the animal body as mentioned below.

Syntheses of aromatic substances with other atomic groups occur frequently. To these syntheses belongs, in the first place, the conjugation of *benzoic acid* with *glycocoll* to form *hippuric acid*, the discovery of which is generally ascribed to WÖHLER, but according to HEFFTER[3] more cor-

[1] Sasaki, Bioch. Zeitschr., **25**; Friedmann, *ibid.*, **35**.

[2] Zeitschr. f. physiol. Chem., **46**.

[3] Die Ausscheidung körperfremder Substanzen im Harn, Ergebnisse der Physiol., **4**, 252.

rectly to KELLER and URE. All the numerous aromatic substances which are converted into benzoic acid in the body are voided partly as hippuric acid. This statement is not true for all species of animals. According to the observations of JAFFÉ,[1] benzoic acid does not pass into hippuric acid in birds, but after conjugation with ornithin, into the corresponding acid, *ornithuric acid*, (α-δ-dibenzoyldiamino valeric acid). Not only are the *oxybenzoic acids* and the *substituted benzoic acids* conjugated with glycocoll, forming corresponding hippuric acids, but also the above-mentioned acids, *toluic*, *mesitylenic*, *cumic*, and *phenylacetic acids*. These acids are voided as *toluric*, *mesitylenuric*, *cuminuric*, and *phenaceturic acids*.

It must be remarked in regard to the oxybenzoic acids that a conjugation with glycocoll has been shown only with salicylic and *p*-oxybenzoic acid (BERTAGNINI, and others), while BAUMANN and HERTER [2] find it only very probable for *m*-oxybenzoic acid. According to BALDONI,[3] in dogs, the salicylic acid does not pass into salicyluric acid, and he indeed found two acids which he calls ursalicylic acid, $C_{14}H_{14}O_8$ and uramin-salicylic acid, $C_{16}H_{16}NO_8$. The oxybenzoic acids are also in part eliminated as conjugated sulphuric acids, which is especially true for *m*-oxybenzoic acid. The three aminobenzoic acids, according to the experiments of HILDEBRANDT, on rabbits, appeared at least in part unchanged in the urine. SALKOWSKI found, as was later confirmed by R. COHN,[4] that in rabbits *m*-aminobenzoic acid passes in part into *uraminobenzoic acid*, $H_2N.CO.HN.C_6H_4.COOH$. It is also in part eliminated as aminohippuric acid.

The behavior of the halogen-substituted compounds of toluene varies in different animals according to HILDEBRANDT'S experiments. In dogs they are converted into the corresponding substituted hippuric acid. In rabbits *o*-bromtoluene is completely changed to hippuric acid, the *m*- and *p*-bromtoluene only partly. The three chlortoluenes are converted in rabbits into the corresponding benzoic acid and are eliminated as such and not as hippuric acid.

The substituted aldehydes are of special interest as substances which may undergo conjugation with glycocoll. According to the investigations of R. COHN [5] on this subject, *o-nitrobenzaldehyde* when introduced into a rabbit is only in a very small part converted into nitrobenzoic acid, and the chief mass, about 90 per cent, is destroyed in the body. According to SIEBER and SMIRNOW [6] *m-nitrobenzaldehyde* passes in dogs into *m*-nitrohippuric acid, and according to COHN into urea-*m*-nitrohippurate, but in rabbits a different action results. In this case not only does an oxidation of the aldehyde into benzoic acid take place, but the

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[1] Ber. d. d. chem. Gesellsch., **10** and **11**.

[2] Zeitschr. f. physiol. Chem., **1**, where Bertagnini's work is also cited. See also Dautzenberg, Maly's Jahresber., **11**, 231.

[3] Arch. f. exp. Path. u. Pharm., 1908, Suppl. Bd. (Schmiedeberg's Festschrift).

[4] Salkowski, Zeitschr. f. physiol. Chem., **7**; Cohn, *ibid.*, **17**; Hildebrandt, Hofmeister's Beiträge, **8**.

[5] Zeitschr. f. physiol. Chem., **17**.

[6] Monatshefte f. Chem., **8**.

nitro-group is also reduced to an amino-group, and finally acetic acid attaches itself to this with the expulsion of water, so that the final product is *m-acetylaminobenzoic acid*, $(CH_3.CO).NH.C_6H_4COOH$. The *p*-nitrobenzaldehyde acts in rabbits in part like the *m*-aldehyde and passes in part into *p-acetylaminobenzoic acid*. Another part is converted into *p*-nitrobenzoic acid, and the urine contains a chemical combination of equal parts of these two acids. According to SIEBER and SMIRNOW *p*-nitrobenzaldehyde yields only urea *p*-nitrohippurate in dogs. The above-mentioned *pyridine-carboxylic acid*, formed from methylpyridine (α-picoline) passes into the urine after conjugation with glycocoll as *α-pyridinuric acid*.[1]

To those substances which undergo a conjugation with glycocoll belongs also *furfurol* (the aldehyde of pyromucic acid, $C_4H_3O.CHO$), which, when introduced into rabbits and dogs, as shown first by JAFFÉ and COHN,[2] and then further shown by SASAKI and FRIEDMANN, is eliminated in two ways from the body. The furfurol can, by a similar synthesis to PERKIN'S reaction, be transformed into the unsaturated acid *furfuracrylic acid* $C_4H_3O.CH:CH.COOH$, and also into *pyromucic acid* $C_4H_3O.COOH$. These two acids pass, after conjugation with glycocoll, into the urine as *furfuracryluric acid* and *pyromucuric acid*. In birds the pyromucic acid is conjugated with ornithine and is eliminated as *pyromucinornithuric acid*.

It has not been proved how *thiophene*, C_4H_4S, behaves in the animal body. Of *methylthiophene* (thiotolene), $C_4H_3S.CH_3$, a very small part is oxidized to thiophenic acid, $C_4H_3S.COOH$ (LEVY). This acid, as shown by JAFFÉ and LEVY,[3] is conjugated with glycocoll in the body (rabbits) and eliminated as *thiophenuric acid*.

Another very important synthesis of aromatic substances is that of the *ethereal-sulphuric acids*. *Phenols*, and in particular the *hydroxylated aromatic hydrocarbons* and their derivatives are voided as ethereal-sulphuric acids, according to BAUMANN, HERTER and others.[4]

A conjugation of aromatic acids with sulphuric acid occurs less often. The two previously mentioned aromatic acids, *p-oxyphenylacetic* and *p-oxyphenylpropionic acid*, are in part eliminated in this form. *Gentisic acid* (hydroquinone-carboxylic acid) also increases, according to LIKHATSCHEFF,[5] the quantity of ethereal-sulphuric acid in the urine, while

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[1] In regard to the extensive literature on glycocoll conjugations we refer the reader to O. Kühling, Ueber Stoffwechselprodukte aromatischer Körper. Inaug.-Diss., Berlin, 1887.

[2] Ber. d. d. Chem. Gesellsch., 20 and 21; Sasaki and Friedmann, footnote 1, page 782.

[3] Levy, Ueber das Verhalten einiger Thiophenderivate, etc., Inaug.-Diss., Königsberg, 1889; Jaffé and Levy, Ber. d. d. chem. Gesellsch., 21.

[4] In regard to the literature, see O. Kühling, l. c.

[5] Zeitschr. f. physiol. Chem., 21.

ROST asserts, contrary to earlier claims, that the same occurs with *gallic acid* (trioxybenzoic acid) and *tannic acid*.[1]

Although NENCKI and REKOWSKI [2] have shown that *acetophenone* (phenylmethylketone), $C_6H_5.CO.CH_3$, is oxidized to benzoic acid and eliminated as hippuric acid, the aromatic oxyketones with hydroxyl groups, such as *resacetophenone*, 2, 4 dioxacetophenone $(HO)_2.C_6H_3.CO.CH_3$, pass into the urine as ethereal-sulphuric acids and in part after conjugation with glucuronic acid. *Euxanthon*, which is also an aromatic ketone, namely dioxyxanthon, $HO.C_6H_3\langle{}^{CO}_{O}\rangle C_6H_3.OH$, passes into the urine as *euxanthic acid* after conjugation with glucuronic acid.

A *conjugation* of other aromatic substances *with glucuronic acid*, which last is protected from combustion, occurs rather often. The phenols, as above stated (page 725), pass in part as conjugated glucuronic acids into the urine. The same is true for the homologues of the phenols, for certain substituted phenols, and for many aromatic substances, also hydrocarbons after previous oxidation and hydration. Thus HILDEBRANDT and FROMM and CLEMENS [3] have shown that the *terpenes* and *camphors*, by oxidation or hydration, or in certain cases by both, are converted into hydroxyl derivatives when the body in question is not previously hydroxylized, and that these hydroxyl derivatives are eliminated as conjugated glucuronic acids. Conjugated glucuronic acids are detected in the urine after the introduction of various substances into the organsim, e.g., therapeutic agents, such as *terpenes*, *borneol*, *menthol*, *camphor* (camphoglucuronic acid was first observed by SCHMIEDEBERG), *naphthalene*, *oil of turpentine*, *oxyquinolines*, *antipyrine*, and many other bodies.[4] *Orthonitrotoluene* in dogs passes first into *o*-nitrobenzyl alcohol and then

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[1] In regard to the behavior of gallic and tannic acids in the animal body, see C. Mörner, Zeitschr. f. physiol. Chem., **16**, which also contains the earlier literature; also Harnack, *ibid.*, **24**, and Rost, Arch. f. exp. Path. u. Pharm., **38**, and Sitzungsber. d. Gesellsch. zur Beförd. d. ges. Naturwiss. zu Marburg, 1898.

[2] Arch. d. scienc. biol. de St. Pétersbourg, **3**, and Ber. d. deutsch. chem. Gesellsch., **27**.

[3] Hildebrandt, Arch. f. exp. Path. u. Pharm., **45**, **46**; Zeitschr. f. physiol. Chem., **36**; with Fromm, *ibid.*, **33**; and with Clemens, *ibid.*, **37**; Fromm and Clemens, *ibid.*, **34**. Extensive investigations on the behavior of alicylic compounds with the glucuronic acid conjugation in the organism have been carried out by Hämäläinen, Skand. Arch. f. Physiol., **27**.

[4] See O. Kühling, l. c., which gives the literature up to 1887; also E. Külz, Zeitschr. f. Biologie, **27**; the works of Hildebrandt, Fromm and Clemens, see footnote 3; Brahm, Zeitschr. f. physiol. Chem., **28**; Fenyvessy, *ibid.*, **30**; Bonanni, Hofmeister's Beiträge, **1**; Lawrow, Ber. d. d. chem. Gesellsch., **33**.

into a conjugated glucuronic acid, *uronitrotoluolic acid* (JAFFÉ [1]). The glucuronic acid split off from this conjugated acid is levogyrate and hence is not identical, but only isomeric, with the ordinary glucuronic acid. *Dimethylaminobenzaldehyde*, according to JAFFÉ, is converted in part into *dimethylaminobenzoglucuronic acid* in rabbits. The same conjugated glucuronic acid is also produced, according to HILDEBRANDT,[2] from *p-dimethyltoluidine*, which is first changed into *p-dimethylaminobenzoic acid*. *Indol* and *skatol* seem, as above stated (page 731), to be eliminated in the urine partly as conjugated glucuronic acids. The mercapturic acids, which will be mentioned below, also belong to those substances which are conjugated with glucuronic acid, and after this conjugation appear in the urine.

A conjugation of *carbamic acid*, NH_2COOH, with *amino-acids* to form *uramino-acids*, R.CH.NH.($CONH_2$)COOH, or their anhydrides, the hydantoins, have also been observed in several cases, as after feeding sarcosin, amino-benzoic acid, phenylalanine, taurine, tyrosine. It must be remarked that according to LIPPICH and DAKIN,[3] the uramino-acids can be easily produced as transformation products from the urea in the concentration of the urine by the aid of heat.

Syntheses with a simultaneous *acetylation* are of great interest. Such a synthesis is the formation of the *mercapturic acids*. These acids, which are produced in the body of dogs after the introduction of brom- and chlorbenzene (BAUMANN and PREUSSE, JAFFÉ, FRIEDMANN [4]) are acetylated derivatives of the protein cystine, and the acetylated brom-phenylcysteine is $CH_2.S(C_6H_4Br).CH.NH(COCH_3).COOH$. Another example of a synthesis with acetylation is the phenylaminoacetic acid, which, as NEUBAUER and WARBURG [5] have shown, in perfusion experiments with dog's livers, gives among other products also acetylated *phenylaminoacetic acid*, $C_6H_5.CHNH(COCH_3).COOH$.

The *synthesis of amino-acids*, with simultaneous acetylation, as recently observed by KNOOP, are of specially great interest. After the introduction of γ-phenyl-α-keto butyric acid into the body of a dog, the formation of the corresponding acetylated amino-acid,

$$C_6H_5.CH_2.CH_2.CHNH(COCH_3).COOH$$

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[1] Zeitschr. f. physiol. Chem., **2**.

[2] Jaffé, Zeitschr. f. physiol. Chem., **43**; Hildebrandt, Hofmeister's Beiträge, **7**.

[3] Lippich, Ber. d. d. chem. Gesellsch., **41**; Dakin, Journ. of biol. Chem., **8**; Weiland, Bioch. Zeitschr., **38**.

[4] Baumann and Preusse, Zeitschr. f. physiol. Chem., **5**; Jaffé, Ber. d. deutsch. chem. Gesellsch., **12**; Friedmann, Hofmeister's Beiträge, **4**.

[5] Neubauer and O. Warburg, Zeitschr. f. physiol. Chem., **70**.

was observed. In perfusion experiments with dog livers, EMBDEN and SCHMITZ [1] have shown the formation of phenylalanine, tyrosine and other amino-acids, as has already been mentioned (pages 530 and 775) by synthesis from the ammonium salts of the corresponding keto-acids and also in part from the oxy-acids.

Methylation also often occurs, and as an example we will mention that HIS has shown that *pyridine*, C_5H_5N, is transformed in dogs into *methylpyridine*, and then passes into the urine as *methylpyridylammonium hydroxide*. Pyridine behaves similarly in hens (HOSHIAI), pigs and goats, (TOTANI and HOSHIAI) while according to ABDERHALDEN [2] and collaborators, in rabbits it passes unchanged into the urine. Further examples of methylation, although not aromatic substances, are the conversion of *guanidine acetic acid* into *creatine* (JAFFÉ) and the observation of TAKEDA [3] of the appearance of *aminobutyrobetaine* in the urine of dogs with phosphorus poisoning.

Several alkaloids, such as *quinine*, *morphine*, and *strychnine*, may pass into the urine. After the ingestion of *turpentine*, *balsam of copaiba*, and *resins*, these may appear in the urine as resin acids. Different kinds of coloring-matters, such as *alizarin*, *crysophanic acid*, after rhubarb or senna, and the *coloring-matter of the blueberry*, etc., may also pass into the urine. After *rhubarb*, *senna*, or *santonin* the urine assumes a yellow or greenish-yellow color, which is transformed into a beautiful red by the addition of alkali. *Phenol* produces, as above mentioned, a dark-brown or dark-green color which depends mainly on the decomposition products of hydroquinone and humin substances. After *naphthalene* the urine has a dark color, and several other medicinal agents produce a special coloration. Thus after antipyrine it becomes yellow or blood-red. After *balsam of copaiba* the urine becomes, when strongly acidified with hydrochloric acid, gradually rose- and purple-red. After *naphthalene* or *naphthol* the urine gives with concentrated sulphuric acid (1 cc. of concentrated acid and a few drops of urine) a beautiful emerald-green color, which is probably due to naphthol-glucuronic acid. Odoriferous bodies also pass into the urine. After asparagus the urine acquires a digusting odor which is probably due to methylmercaptan. After turpentine the urine may have a peculiar odor similar to that of violets.

VI. PATHOLOGICAL CONSTITUENTS OF URINE.

Proteid. The appearance of slight traces of proteid in normal urine has been observed by many investigators, such as POSNER PLÓSZ, v. NOORDEN, LEUBE, and others. According to K. MÖRNER [4] proteid regularly occurs as a normal urinary constituent to the extent of 22–78

[1] Knoop, Zeitschr. f. physiol. Chem., **67**; Embden and Schmitz, Bioch. Zeitschr., **29** and **38**.

[2] His, Arch. f. exp. Path. u. Pharm., **22**; Cohn, Zeitschr. f. physiol. Chem., 18; Hoshiai, *ibid.*, **62**; with Totani, *ibid.*, **68**; Abderhalden and collaborators, *ibid.*, **59** and **62**.

[3] Jaffé, Zeitschr. f. physiol. Chem., **48**; Takeda, Pflüger's Arch., **133**.

[4] Skand. Arch. f. Physiol., **6** (literature).

milligrams per liter. Frequently traces of a substance similar to a nucleoalbumin, which is easily mistaken for mucin, and whose nature will be treated of later, appears in the urine. In diseased conditions proteid occurs in the urine in a variety of cases. The albuminous bodies which most often occur are serglobulin and seralbumin. Proteoses (or peptones) are also sometimes present. The quantity of proteid in the urine is in most cases less than 5 p. m., rarely 10 p. m., and only very rarely does it amcunt to 50 p. m. or over. Cases are known, however, where it was even more than 80 p. m.

Among the many reactions proposed for the detection of proteid in urine, the following are to be recommended:

The Heat Test. Filter the urine and test its reaction. An acid urine may, as a rule, be boiled without further treatment, and only in especially acid urines is it necessary to first treat with a little alkali. An alkaline urine is made neutral or faintly acid before heating. If the urine is poor in salts, add 1/10 vol. of a saturated common-salt solution before boiling; then heat to the boiling-point, and if no precipitation, cloudiness, or opalescence appears, the urine in question contains no coagulable proteid, but it may contain proteoses or peptones. If a precipitate is produced on boiling, this may consist of proteid, or of earthy phosphates,[1] or of both. The monohydrogen calcium phosphate decomposes on boiling, and the normal phosphate may separate out. The proper amount of acid is now added to the urine, so as to prevent any mistake caused by the presence of earthy phosphates, and to give a better and more flocculent precipitate of the proteid. If acetic acid is used for this, then add 1–3 drops of a 25 per cent acid to each 10 cc. of the urine and boil after the addition of each drop. On using nitric acid, add 1–2 drops of the 25 per cent acid to each cubic centimeter of the boiling-hot urine.

On using acetic acid, when the quantity of proteid is very small, and especially when the urine was originally alkaline, the proteid may sometimes remain in solution on the addition of the above quantity of acid. If, on the contrary, less acid is added, the precipitate of calcium phosphate, which forms in amphoteric or faintly acid urines, is liable not to dissolve completely, and this may cause it to be mistaken for a proteid precipitate. If nitric acid is used for the heat test, the fact must not be overlooked that after the addition of only a little acid a combination between it and the proteid is formed which is soluble on boiling and which is only precipitated by an excess of the acid. On this account the large quantity of nitric acid, as suggested above, must be added, but in this case a small part of the proteid is liable to be dissolved by the excess of the nitric acid. When the acid is added after boiling, which is absolutely necessary, the liability of a mistake is not so great. It is on these grounds that the heat test, although it gives very good results in the hands of experts, is not recommended to physicians as a positive test for proteid.

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[1] In regard to the cause of the phosphate precipitation on boiling the urine, see Malfatti, Hofmeister's Beiträge, 8.

A confounding with mucin, when this body occurs in the urine, is easily prevented in the heat test with acetic acid by acidifying another portion with acetic acid at the ordinary temperature. Mucin and nucleoalbumin substances similar to mucin are hereby precipitated. If in the performance of the heat and nitric-acid test, a precipitate first appears on cooling or is strikingly increased, then this shows the presence of proteoses in the urine, either alone or mixed with coagulable proteid. In this case a further investigation is necessary (see below). In a urine rich in urates a precipitate consisting of uric acid separates on cooling. This precipitate is colored and granular, and is hardly to be mistaken for a proteose or proteid precipitate.

HELLER'S *test* is performed as follows (see page 99): The urine is very carefully floated on the surface of nitric acid in a test-tube, or the urine is placed in a test-tube and then the acid is slowly added by means of a funnel, drawn out to a point, and extending to the bottom. In the presence of albumin a white disk, or as we ordinarily say a white ring or at least a sharply defined cloudiness, appears at the point of contact of the two fluids. With this test a red or reddish-violet transparent ring is always obtained with normal urine; it depends upon the indigo coloring-matters and can hardly be mistaken for the white or whitish proteid ring. In a urine rich in urates, another complication may occur, due to the formation of a ring produced by the precipitation of uric acid. The uric-acid ring does not lie, like the proteid ring, between the two liquids, but somewhat higher. For this reason two simultaneous rings may exist in urines which are rich in urates and do not contain very much proteid. The disturbance caused by uric acid is easily prevented by diluting the urine with 1–2 vols. of water before performing the test. The uric acid now remains in solution, and the delicacy of HELLER'S test is so great that after dilution only in the presence of insignificant traces of proteid does this test give negative results. In a urine very rich in urea a ring-like separation of urea nitrate may also appear. This ring consists of shining crystals, and it does not appear in urine previously diluted. A confusion with resinous acids, which also give a whitish ring with this test, is easily prevented, since these acids are soluble on the addition of ether. Stir, add ether, and carefully shake the contents of the test-tube. If the cloudiness is due to resinous acids, the urine gradually becomes clear, and on evaporating the ether a sticky residue of resinous acids is obtained. A liquid which contains true mucin does not give a precipitate with this test, but it gives a more or less strongly opalescent ring, which disappears on stirring. The liquid does not contain any precipitate after stirring, but is somewhat opalescent. If a faint, not wholly typical reaction is obtained with HELLER'S test after some time with undiluted urine, while the diluted urine gives a pronounced reaction, the presence is shown of the substance which used to be called mucin or nucloealbumin. In this case proceed as described below for the detection of nucleoalbumin.

If the above-mentioned possible errors and the means by which they may be prevented are borne in mind, there is hardly another test for proteid in the urine which is at the same time so easily performed, so delicate, and so positive as HELLER'S. With this test even 0.002 per cent of albumin may be detected without difficulty. Still the student must not be satisfied with this test alone, but should apply at least a

second one, such as the heat test. In performing this test the (primary) proteoses are also precipitated.

The *reaction with metaphosphoric acid* is very convenient and easily performed. It is not quite so delicate and positive as HELLER'S test. The proteoses are also precipitated by this reagent.

Reaction with Acetic Acid and Potassium Ferrocyanide. Treat the urine first with acetic acid until it contains about 2 per cent, and then add drop by drop a potassium-ferrocyanide solution (1:20), carefully avoiding an excess. This test is very good, and in the hands of experts it is even more delicate than HELLER'S. In the presence of a very small quantity of proteid it requires more practice and dexterity than HELLER'S, as the relative quantities of reagent, proteid, and acetic acid influence the result of the test. The quantity of salts in the urine likewise seems to have an influence. This reagent also precipitates proteoses.

SPIEGLER'S *Test.* SPIEGLER recommends a solution of 8 parts mercuric chloride, 4 parts tartaric acid, 20 parts glycerin, and 200 parts water as a very delicate reagent for proteid in the urine. A test-tube is half filled with this reagent and the urine is allowed to flow upon its surface drop by drop from a pipette along the wall of the test-tube. In the presence of proteid a white ring is obtained at the point of contact between the two liquids. The delicacy of this test is 1:350,000. JOLLES [1] does not consider this reagent suited for urines very poor in chlorine, and for this reason he has changed it as follows: 10 grams mercuric chloride, 20 grams succinic acid, 10 grams NaCl, and 500 cc. water.

Reaction with *sulphosalicylic acid.* Treat the urine either with a 20 per cent watery solution of sulphosalicylic acid or a few crystals of the acid. This reagent does not precipitate the uric acid or the resin acids. (ROCH'S [2] test.)

As every normal urine contains traces of proteid, it is apparent that very delicate reagents are to be used only with the greatest caution. For ordinary cases HELLER'S test is sufficiently delicate. If no reaction is obtained with this test within 2½ to 3 minutes, the urine tested contains less than 0.003 per cent of proteid, and is to be considered free from proteid in the ordinary sense.

The use of precipitating reagents presumes that the urine to be investigated is perfectly clear, especially in the presence of only very little proteid. The urine must first be filtered. This is not easily done with urine containing bacteria, but a clear urine may be obtained, as suggested by A. JOLLES, by shaking the urine with infusorial earth. Although a little proteid is retained in this procedure and lost, it does not seem to be of any importance (GRÜTZNER, SCHWEISSINGER [3]).

The different *color reactions* cannot be directly used, esspecially in deep-colored urines which contain only little proteid. The common salt of the urine has a disturbing action on MILLON'S reagent. To prove more positively the presence

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[1] Spiegler, Wien. klin. Wochenschr., 1892, and Centralbl. f. d. klin. Med., 1893; Jolles, Zeitschr. f. physiol. Chem., **21**.

[2] Pharmaceut. Centralbl., 1889, and Zeitschr. f. anal. Chem., **29**.

[3] Jolles, Zeitschr. f. anal. Chem., **29**; Grützner, Chem. Centralbl., 1901, **1**; Schweissinger, *ibid.*

of protein, the precipitate obtained in the boiling test may be filtered, washed, and then tested with MILLON'S reaction. The precipitate may also be dissolved in dilute alkali and the biuret test applied to the solution. The presence of proteoses or peptones in the urine is directly tested for by this last-mentioned test.

In testing the urine for proteid one should never be satisfied with one reaction alone, but must apply the heat test and HELLER'S, or the potassium-ferrocyanide test. In using the heat test alone the proteoses may be easily overlooked, but these are detected, on the contrary, by HELLER'S or the potassium-ferrocyanide test. If only one of these tests is employed, no sufficient intimation of the kind of proteid present can be obtained, whether it consists of proteoses or coagulable proteid.

For practical purposes several dry reagents for proteid have been recommended. Besides the metaphosphoric acid may be mentioned STUTZ'S or FÜRBRINGER'S gelatin capsules, which contain mercuric chloride, sodium chloride, and citric acid; and GEISSLER'S albumin-test papers, which consist of strips of filter-paper some of which have been dipped in a solution of citric acid, and some into a solution of mercuric-chloride and potassium-iodide solution, and then dried.

If the presence of proteid has been positively proved in the urine by the above tests, it then remains necessary to determine its character.

The *Detection of Globulin and Albumin.* In detecting serglobulin the urine is exactly neutralized, filtered, and treated with magnesium sulphate in substance until it is completely saturated at the ordinary temperature, or with an equal volume of a saturated neutral solution of ammonium sulphate. In both cases a white, flocculent precipitate is formed in the presence of globulin. In using ammonium sulphate with a urine rich in urates, a precipitate consisting of ammonium urate may separate. This precipitate does not appear immediately, but only after a certain time, and it must not be mistaken for the globulin precipitate. In detecting seralbumin heat the filtrate from the globulin precipitate to boiling-point, or add about 1 per cent acetic acid to it at the ordinary temperature.

For the detection and also for the quantitative estimation of the various globulins (fibringlobulin, euglobulin, and pseudoglobulin) OSWALD [1] has proposed the fractional precipitation with ammonium sulphate.

Proteoses and *peptones* have been repeatedly found in the urine in different diseases. Reliable reports are at hand on the occurrence of proteoses in the urine. The statements in regard to the occurrence of peptones date from a time when the conception of proteoses and peptones was different from that of the present day, and in part they are based upon investigations using untrustworthy methods. According to ITO [2] true peptones are sometimes found in the urine in cases of pneu-

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[1] Münch. med. Wochenschr., 1904. See also Zak and Necker, Deutsch. Arch. f. klin. Med., 88.

[2] In regard to the literature on proteoses and peptones in urine, see Huppert-Neubauer, Harn-Analyse, 10. Aufl., 466 to 492; also A. Stoffregen, Ueber das Vorkommen von Pepton im Harn, Sputum, und Eiter (Inaug.-Diss., Dorpat, 1891); E. Hirschfeldt, Ein Beitrage zur Frage der Peptonurie (Inaug.-Diss., Dorpat, 1892); and espe-

monia; what has been designated as urine peptones seems to have been chiefly deuteroproteoses.

In detecting the proteoses, the proteid-free urine, or urine boiled with addition of acetic acid, is saturated with ammonium sulphate, which precipitates the proteoses. Several errors are here possible. The urobilin, which may give a reaction similar to the biuret reaction, is also precipitated and may lead to mistakes (SALKOWSKI, STOKVIS [1]). The following modification by BANG and DEVOTO'S [2] method can be used to advantage: The urine is heated to boiling with ammonium sulphate (8 parts to 10 parts urine) and boiled for a few seconds. The hot liquid is centrifuged for ½ to 1 minute and separated from the sediment. The urobilin is removed from this by extraction with alcohol. The residue is suspended in a little water, heated to boiling, filtered, whereby the coagulable proteid is retained on the filter, and any urobilin still present in the filtrate is shaken out with chloroform. The watery solution, after removal of the chloroform, is used for the biuret test. For clinical purposes this method is very serviceable.

According to SALKOWSKI the urine treated with 10-per cent hydrochloric acid is precipitated with phosphotungstic acid, then warmed, the liquid decanted from the resin-like precipitate, this washed with water, and then dissolved in a little water with the aid of some caustic soda, warmed again until the blue color disappears, cooled, and finally tested with copper sulphate. This method has been somewhat modified by v. ALDOR and CERNY.[3] In regard to other more complicated methods we refer to HUPPERT-NEUBAUER.

MORAWITZ and DIETSCHY [4] first remove the proteid from the urine made faintly acid with acid potassium phosphate by the addition of double the volume of 96-per cent alcohol and warming on the water-bath for several hours. From the concentrated filtrate acidified with a little sulphuric acid the proteoses can be precipitated by saturating with zinc sulphate. After the removal of the urobilin by alcohol and extracting with water, the biuret test may be applied.

If the proteoses have been precipitated from a larger portion of urine by ammonium sulphate, this precipitate is tested for the presence of different proteoses for the reasons given in Chapter II. The following serves as a preliminary determination of the character of the proteoses present in the urine. If the urine contains only deuteroproteose it does not become cloudy on boiling, does not give HELLER'S test, does not become cloudy on saturating with NaCl in neutral reaction, but does become turbid on adding acetic acid saturated with this salt. In the presence of only protoproteose the urine gives HELLER'S test, is precipitated even in neutral solution on saturating with NaCl, but does not coagulate on boiling. The presence of heteroproteose is shown by the urine behaving like the above with NaCl and nitric acid, but shows a difference on heating. It gradually becomes cloudy on warming and separates at about 60° C. a sticky precipitate which attaches itself to the sides of the vessel and which dissolves at boiling temperature on acidifying the urine; the precipitate reappears on cooling.

In close relation to the proteoses stands the so-called BENCE-JONES proteid, which occurs in the urine in rare cases in diseases with changes

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cially Stadelmann, Untersuchungen über die Peptonurie, Wiesbaden, 1894; Ehrström, Bidrag till kännedomen om Albumosurien, Helsingfors, 1900; Ito, Deutsch. Arch. f. klin. Med., **71**.

[1] Salkowski, Berlin. klin. Wochenschr., 1897; Stokvis, Zeitschr. f. Biologie, **34**.

[2] Devoto, Zeitschr. f. physiol. Chem., **15**; Bang, Deutsch. med. Wochenschr., 1898.

[3] Salkowski, Centralbl. f. d. med. Wissensch., 1894; v. Aldor, Berl. klin. Wochenschr., **36**; Černy, Zeitschr. f. analyt. Chem., **40**.

[4] Arch. f. exp. Path. u. Pharm., **54**.

in the spinal marrow. It gives a precipitate on heating to 40–60° C., which on further heating to boiling dissolves again more or less completely, depending upon the reaction and upon the amount of salt present. In salt-free solution the precipitate is not dissolved, on heating to boiling, at least not always. It does not separate on dialysis, but can be precipitated from the urine by double the volume of a saturated ammonium-sulphate solution or by alcohol. It has also been obtained as crystals (GRUTTERINK and DE GRAAFF, MAGNUS-LEVY [1]). This body shows a varying behavior in the different cases in which it has been found and its nature has not been explained. From the investigations of the above-mentioned and other experimenters (MOITESSIER, ABDERHALDEN and ROSTOSKI) we can draw the conclusion that this proteid is similar to the proteoses in several reactions, but that nevertheless it stands close to the genuine protein bodies. It also yields primary as well as secondary proteoses on peptic digestion (GRUTTERINK and DE GRAAFF), and yields the same hydrolytic cleavage products as the other proteins (ABDERHALDEN and ROSTOSKI).

Quantitative Estimation of Proteid in Urine. Of all the methods proposed thus far, the COAGULATION METHOD (boiling with the addition of acetic acid) when performed with sufficient care gives the best results. The average error need never amount to more than 0.01 per cent, and it is generally smaller. With this method it is best to first find how much acetic acid must be added to a small portion of the urine, which has been previously heated on the water-bath, to completely separate the proteid so that the filtrate will not respond to HELLER'S test. Then coagulate 20–50–100 cc. of the urine. Pour the urine into a beaker and heat on the water-bath, add the required quantity of acetic acid slowly, stirring constantly, and heat at the same time, Filter while warm, wash first with water, then with alcohol and ether, dry and weigh, incinerate and weigh again. In exact determinations the filtrate must not give HELLER'S test.

The separate estimation of GLOBULINS and ALBUMINS is done by carefully neutralizing the urine and precipitating with $MgSO_4$ added to saturation (HAMMARSTEN), or simply by adding an equal volume of a saturated neutral solution of ammonium sulphate (HOFMEISTER and POHL [2]). The precipitate consisting of globulin is thoroughly washed with a saturated magnesium-sulphate or half-saturated ammonium-sulphate solution, dried continuously at 110° C., boiled with water, extracted with alcohol and ether, then dried, weighed, incinerated, and weighed again. The quantity of albumin is calculated as the difference between the quantity of globulin and the total proteids.

Approximate Estimation of Proteid in Urine. Of the methods suggested for this purpose none has been more extensively employed than ESBACH'S.

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[1] Magnus-Levy, Zeitschr. f. physiol. Chem., **30** (literature); Grutterink and de Graaff, *ibid.*, **34** and **36**; Moitessier, Compt. rend. soc. biolog., **57**; Ville and Derrien, *ibid.*, **62**; Abderhalden and Rostoski, Zeitschr. f. physiol Chem., **46**; see also Hopkins and Savory, Journ. of Physiol., **42**.

[2] Hammarsten, Pflüger's Arch., **17**; Hofmeister and Pohl, Arch. f. exp. Path. u. Pharm., **20**.

ESBACH'S [1] *Method.* The acidified urine (with acetic acid) is poured into a specially graduated tube to a certain mark, and then the reagent (a 2-per cent citric-acid and 1 per cent picric-acid solution in water) is added to a second mark, the tube closed with a rubber stopper and carefully shaken, avoiding the production of froth. The tube is allowed to stand twenty-four hours, and then the height of the precipitate on the graduation is read off. The reading gives directly the quantity of proteid in 1000 parts of the urine. Urines rich in proteid must first be diluted with water. The results obtained by this method, are, however, dependent upon the temperature; and a difference in temperature of 5° to 6.5° C. may cause an error of 0.2–0.3 per cent deficiency or excess in urines containing a mediun quantity of proteid (CHRISTENSEN and MYGGE). The method suggested by TSUSCHIJA [2] seems to be more reliable, and consists in precipitating the proteid by an alcoholic solution of phosphotungstic acid containing hydrochloric acid.

Other methods for the approximate estimation of proteid are the optical methods of CHRISTENSEN and MYGGE, and of WALBUM,[3] of ROBERTS and STOLLNIKOW as modified by BRANDBERG, with HELLER'S test, which has been simplified for practical purposes by MITTELBACH. The density methods of LANG, HUPPERT and ZAHOR are also very good. In regard to these and other methods we refer to HUPPERT-NEUBAUER'S Harn-Analyse, 10. Aufl.

There is at present no trustworthy method for the quantitative estimation of proteoses and peptone in the urine.

Nucleoalbumin and Mucin. According to K. MÖRNER traces of urinary mucoids may pass into solution in the urine; otherwise normal urine contains no mucin. There is no doubt that there may be cases where true mucin appears in the urine; in most cases mucin has probably been mistaken for so-called nucleoalbumin. The occurrence, under some circumstances, of nucleoalbumin in the urine is not to be denied, as such substances occur in the renal and urinary passages; still in most cases this nucleoalbumin, as shown by K. MÖRNER,[4] is of an entirely different kind.

All urine, according to MÖRNER, contains a little proteid and in addition substances which precipitate proteid. If the urine freed from salts by dialysis is shaken with chloroform after the addition of 1-2 p. m. acetic acid, a precipitate is obtained which acts like a nucleoalbumin. If the acid filtrate is treated with seralbumin, a new and similar precipitate is obtained, due to the presence of a residue of the substance which precipitates proteids. The most important of these proteid-precipitating substances is chondroitin-sulphuric acid and nucleic acid, although the latter appears to a much smaller extent. Taurocholic acid may in a few instances, especially in icteric urines, be precipitated. The substances isolated by different investigators from urine by the addition of acetic acid and called "dissolved mucin" or "nucleoalbumin" are considered

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[1] In regard to the literature on this method and the numerous experiments to determine its value, see Huppert-Neubauer, 10 Aufl., 853 and Neuberg, Der Harn, s. 765.

[2] Christensen, Virchow's Arch., 115; Tsuschija, Centralbl. f. Med., 1908.

[3] Deutsch. med. Wochenschrift, 1908.

[4] Skand. Arch. f. Physiol., 6.

by MÖRNER to be a combination of proteid chiefly with chondroitin-sulphuric acid, and to a less extent with nucleic acid, and also perhaps with taurocholic acid.

As normal urine habitually contains an excess of substances capable of precipitating proteids, it is apparent that an increased elimination of so-called nucleoalbumin may be caused simply by an augmented excretion of proteid. This happens to a still greater extent in cases where the proteid as well as the proteid-precipitating substance is eliminated to an increased extent.

Detection of so-called Nucleoalbumins. When a urine becomes cloudy or precipitates on the addition of acetic acid, and when it gives a more typical reaction with HELLER'S test after the dilution of the urine than before, one is justified in making tests for mucin and nucleoalbumin. As the salts of the urine interfere considerably with the precipitation of these substances by acetic acid, they must first be removed by dialysis. As large a quantity of urine as possible is dialyzed (with the addition of chloroform) until the salts are removed. The acetic acid is added until it contains 2 p. m., and the mixture allowed to stand. The precipitate is dissolved in water by the aid of the smallest possible quantity of alkali and precipitated again. In testing for chrondroitin-sulphuric acid a part is warmed on the water-bath with about 5 per cent hydrochloric acid. If positive results are obtained on testing for sulphuric acid and reducing substance, then chondroproteid was present. If a reducing substance can be detected but no sulphuric acid, then mucin is probably there. If it does not contain any sulphuric acid or reducing substance, a part of the precipitate is exposed to pepsin digestion and another part used for the determination of any organic phosphorus. If positive results are obtained from these tests, then nucleoalbumin and nucleoproteid must be differentiated by special tests for nuclein bases. No positive conclusion can be drawn except by using very large quantities of urine. The filtrate from the nucleoalbumin can be used for the ordinary proteid tests.

Nucleohistone. In a case of pseudoleucæmia A. JOLLES found a phosphorized protein substance which he considers as identical with nucleohistone. *Histone* is claimed to have been found in some cases by KREHL and MATTHES, and by KOLISCH and BURIAN.[1]

The nitrogen contained in the substances precipitated by alcohol, called the "*colloidal nitrogen*" by SALKOWSKI and whose quantity is doubled in carcinoma as compared to the normal, consists in great part of oxyproteic acids. According to SALKOWSKI and KOJO [2] this can be precipitated by basic lead acetate and the nitrogen determined therein.

Blood and Blood-coloring Matters. The urine may contain blood from hemorrhage in the kidneys or other parts of the urinary passages (HÆMA-

[1] Jolles, Ber. d. deutsch. chem. Gesellsch., **30**; Krehl and Matthes, Deutsch. Arch. f. klin. Med., **54**; Kolisch and Burian, Zeitschr. f. klin. Med., **29**.

[2] Salkowski, Berl. klin. Wochenschr., 1905 and 1910; Kojo, Zeitschr. f. physiol. Chem., **73**.

TURIA). In these cases, when the quantity of blood is not very small, the urine is more or less cloudy and colored reddish, yellowish red, dirty red, brownish red, or dark brown. In recent hemorrhages in which the blood has not decomposed the color is nearer blood-red. Blood-corpuscles may be found in the sediment, sometimes also blood-casts and smaller or larger blood-clots.

In certain cases the urine contains no blood-corpuscles, but only dissolved blood-coloring matters, hæmoglobin, or, and indeed quite often, methæmoglobin (HÆMOGLOBINURIA). The blood-pigments appear in the urine under different conditions, as in dissolution of blood in poisoning with arseniuretted hydrogen, chlorates, etc., after serious burns, after transfusion of blood, and also in the periodic appearance of hæmoglobinuria with fever. In hæmoglobinuria the urine may also have an abundant grayish-brown sediment rich in proteid which contains the remains of the stromata of the red blood-corpuscles. In animals, hæmoglobinuria may be produced by many causes which force free hæmoglobin into the plasma.

To detect blood in the urine, we make use of the microscope, the spectroscope, the guaiac test, and HELLER'S or HELLER-TEICHMANN'S test.

Microscopic Investigation. The blood-corpuscles may remain undissolved for a long time in acid urine; in alkaline urine, on the contrary, they are easily changed and dissolved. They often appear entirely unchanged in the sediment; in some cases they are distended and in others unequally pointed or jagged like a thorn-apple. In hemorrhage of the kidneys a cylindrical clot is sometimes found in the sediment which is covered with numerous red blood-corpuscles, forming casts of the urinary passages. These formations are called *blood-casts*.

The *spectroscopic investigation* is naturally of very great value; and if it be necessary to determine not only the presence but also the kind of coloring-matter, this method is indispensable. In regard to the optical behavior of the various blood-pigments we must refer to Chapter V.

Guaiac Test. Mix in a test-tube equal volumes of tincture of guaiac and old turpentine which has become strongly ozonized by the action of air under the influence of light. To this mixture, which must not have the slightest blue color, add the urine to be tested. In the presence of blood or blood-pigments, first a bluish-green and then a beautiful blue ring appears where the two liquids meet. On shaking the mixture it becomes more or less blue. Normal urine or one containing proteid does not give this reaction. According to LIEBERMANN[1] this reaction is brought about by the blood pigments acting as catalyst upon the organic peroxides existing in the turpentine, accelerating the decomposition of these and the active oxygen taken up by the guaiaconic acid which is oxidized to guaiac blue (guaiaconic acid ozonide). Urine con-

[1] Pflüger's Arch., 104.

taining pus, even when no blood is present, gives a blue color with these reagents; but in this case the tincture of guaiac alone, without turpentine, is colored blue by the urine (VITALI [1]). This is at least true for a tincture that has been exposed for some time to the action of air and sunlight. The blue color produced by pus differs from that produced by blood-coloring matters by disappearing on heating the urine to boiling. A urine alkaline by decomposition must first be made faintly acid before performing the reaction. The turpentine should be kept exposed to sunlight, while the tincture of guaiac must be kept in a dark glass bottle. These reagents to be of use must be controlled by a liquid containing blood. With positive results, however, this test is not absolutely decisive, because other bodies may give a similar reaction, but when properly performed it is so extremely delicate that when it gives negative results any other test for blood is superfluous.[2]

As the delicacy of the above-mentioned tests is sufficient for ordinary purposes it is not necessary to give the new blood-tests suggested recently.

HELLER-TEICHMANN'S *Test.* If a neutral or faintly acid urine containing blood is heated to boiling, one always obtains a mottled precipitate consisting of proteid and hæmatin. If caustic soda is added to the boiling-hot test, the liquid becomes clear and turns green when examined in thin layers (due to hæmatin alkali), and a red precipitate, appearing green by reflected light, re-forms, consisting of earthy phosphates and hæmatin. This reaction is called HELLER'S blood-test. If this precipitate is after a time collected on a small filter, it may be used for the hæmin test (see page 293). If the precipitate contains only a little blood-coloring matter with a larger quantity of earthy phosphates, then wash it with dilute acetic acid, which dissolves the earthy phosphates, and use the residue for the preparation of TEICHMANN'S hæmin crystals. If, on the contrary, the amount of phosphates is very small, then first add a little $MgCl_2$ solution to the urine, heat to boiling, and add simultaneously with the caustic potash some sodium-phosphate solution. In the presence of only very small quantities of blood, first make the urine very faintly alkaline with ammonia, add tannic acid, acidify with acetic acid, and use this precipitate in the preparation of the hæmin crystals (STURVE [3]).

O. and R. ADLER [4] have recommended leucomalachite green or benzidine in the presence of peroxide and acetic acid as especially sensitive reagents for blood.

Hæmatoporphyrin. Since the occurrence of hæmatoporphyrin in the urine in various diseases has been made very probable by several investigators, such as NEUSSER, STOKVIS, MACMUNN, LE NOBEL, COPEMAN, and others,[5] SALKOWSKI has positively shown the presence of this pigment in the urine after sulfonal intoxication. It was first isolated in a pure

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[1] See Maly's Jahresber., 18.

[2] For more details in regard to the preparation of the reagents and the performance of the reaction see O. Schumm., Zeitschr. f. physiol. Chem., 50.

[3] Zeitschr. f. anal. Chem., 11.

[4] Zeitschr. f. physiol. Chem., 41.

[5] A very complete index of the literature on hæmatoporphyrin in the urine may be found in R. Zoja, Su qualche pigmento di alcune urine, etc., in Arch. Ital. di. clin. Med., 1893.

crystalline state by HAMMARSTEN [1] from the urine of insane women after sulfonal intoxication. According to GARROD and SAILLET [2] traces of hæmatoporphyrin (SAILLET'S urospectrin) regularly occur in normal urines. It is also found in the urine during different diseases. It was found in great abundance in a case of typhoid fever (ARNOLD [3]) but otherwise it generally occurs only in small amounts. It has been found in considerable quantities in the urine after the lengthy use of sulfonal.

Urine containing hæmatoporphyrin is sometimes only slightly colored, while in other cases, as for example, after the use of sulfonal, it is more or less deep red. In these last-mentioned cases the color depends, in greatest part, not upon the hæmatoporphyrin, but upon other red or reddish-brown pigments which have not been sufficiently studied.

In the detection of small quantities of hæmatoporphyrin proceed as suggested by GARROD. Precipitate the urine with a 10-per cent caustic-soda solution (20 cc. for every 100 cc. of urine). The phosphate precipitate containing the pigment is dissolved in alcohol-hydrochloric acid (15–20 cc.) and the solution investigated with the spectroscope. In more exact investigations make the solution alkaline with ammonia, add enough acetic acid to dissolve the phosphate precipitate, shake with chloroform, which takes up the pigment, and test this solution with the spectroscope.

In the presence of larger quantities of hæmatoporphyrin the urine is first precipitated, according to SALKOWSKI, with an alkaline barium-chloride solution (a mixture of equal volumes of barium-hydroxide solution, saturated in the cold, and a 10-per cent barium-chloride solution), or, according to HAMMARSTEN,[4] with a barium-acetate solution. The washed precipitate, which contains the hæmatoporphyrin, is allowed to stand some time at the temperature of the room, with alcohol containing hydrochloric or sulphuric acid, and then filtered. The filtrate shows the characteristic spectrum of hæmatoporphyrin in acid solution and gives the spectrum of alkaline hæmatoporphyrin after saturation with ammonia. If the alcoholic solution is mixed with chloroform and a large quantity of water added and carefully shaken, sometimes a lower layer of chloroform is obtained which contains very pure hæmatoporphyrin, while the upper layer of alcohol and water contains the other pigments besides some hæmatoporphyrin.

Other methods which have no advantage over this one of GARROD have been suggested by RIVA and ZOJA as well as SAILLET.[5]

BAUMSTARK [6] found in a case of leprosy two characteristic coloring-matters in the urine, "urorubrohæmatin" and "urofuscohæmatin," which, as their

[1] Salkowski, Zeitschr. f. physiol. Chem., 15; Hammarsten, Skand. Arch. f. Physiol., 8.

[2] Garrod, Journ. of Physiol., 13 (contains review of literature) and 17; Saillet, Revue de Médecine, 16.

[3] Zeitschr. f. physiol. Chem., 82.

[4] Salkowski, l. c.; Hammarsten, l. c.

[5] Riva and Zoja, Maly's Jahresber., 24; Saillet, l. c. See also Nebelthau, Zeitschr. f. physiol. Chem., 27.

[6] Pflüger's Arch., 9.

names indicate, seem to stand in close relation to the blood-coloring matters. *Urorubrohæmatin*, $C_{68}H_{94}N_8Fe_2O_{22}$, contains iron and shows in acid solution an absorption-band in front of *D* and a broader one back of *D*. In alkaline solution it shows four bands—behind *D*, at *E*, beyond *F*, and behind *G*. It is not soluble either in water, alcohol, ether, or chloroform. It gives a beautiful brownish-red non-dichroic liquid with alkalies. *Urofuscohæmatin*, $C_{68}H_{106}N_8O_{22}$, which is free from iron, shows no characteristic spectrum; it dissolves in alkalies, producing a brown color. It remains to be proven whether these two pigments are related to (impure) hæmatoporphyrin.

Melanin. In the presence of melanotic cancers dark pigments are sometimes eliminated with the urine. K. MÖRNER has isolated two pigments from such a urine, of which one was soluble in warm 50-75 per cent acetic acid, while the other, on the contrary, was insoluble. The one seemed to be *phymatorhusin* (see Chapter XV). Usually the urine does not contain any melanin, but a chromogen of melanin, a *melanogen*. In such cases the urine gives EISLET'S reaction, becoming dark-colored with oxidizing agents, such as concentrated nitric acid, potassium bichromate, and sulphuric acid, as well as with free sulphuric acid. They also give THORMÄHLEN'S reaction namely a beautiful blue coloration with sodium nitroprusside and then acetic acid. Urine containing melanin or melanogen is colored black by a ferric-chloride solution (v. JAKSCH [1]).

In a case of melanotic sarcoma H. EPPINGER [2] has isolated from the urine a crystalline melanogen of the composition $C_6H_{12}N_2SO_4$, and which was insoluble in ether. It gave the ordinary melanogen reactions and, according to him is probably an amidated ethereal sulphuric acid of methylpyrrolidinoxycarboxylic acid, which is derived from tryptophane.

Pus occurs in the urine in various inflammatory affections, especially in catarrh of the bladder and in inflammation of the pelvis of the kidneys, or of the urethra.

Pus is best detected by means of the miscroscope. The pus-cells are rather easily destroyed in alkaline urines. In detecting pus we make use of DONNÉ'S pus test, which is performed in the following way: Pour off the urine from the sediment as carefully as possible, place a small piece of caustic alkali on the sediment, and stir. If the pus-cells have not been previously changed, the sediment is converted by this means into a slimy tough mass.

The pus-corpuscles swell up in alkaline urines, and dissolve, or at least are so changed that they cannot be recognized under the microscope. The urine in these cases is more or less slimy or fibrous, and the proteid can be precipitated in large flakes by acetic acid, so that it might possibly be mistaken for mucin. The closer investigation of the precipitate produced by acetic acid, and especially the appearance or non-appearance of a reducing substance after boiling it with a mineral acid, demonstrates the nature of the precipitated substance. Urine containing pus always contains proteid.

Bile-acids. The reports in regard to the occurrence of bile-acids in the urine under physiological conditions do not agree. According to DRAGENDORFF and HÖNE traces of bile-acids occur in the urine; according to MAC-

[1] Thormählen, Virchow's Arch., 108; v. Jaksch, Zeitschr. f. physiol. Chem., 13.

[2] Bioch. Zeitschr., 28.

KAY and v. UDRÁNSZKY and K. MÖRNER[1] they do not. Pathologically they are present in the urine in hepatogenic icterus, although not invariably.

Detection of Bile-acids in the Urine. PETTENKOFER'S test gives the most decisive reaction; but as it gives similar color reactions with other bodies, it must be supplemented by the spectroscopic investigation. The direct test for bile-acids is easily performed after the addition of traces of bile to a normal urine. But the direct detection in a colored icteric urine is more difficult and gives very misleading results; the bile-acid must therefore always be isolated from the urine. This may be done by the following method of HOPPE-SEYLER, which is slightly modified in non-essential points.

HOPPE-SEYLER'S *Method.* Concentrate the urine and extract the residue with strong alcohol. The filtrate is freed from alcohol by evaporation and then precipitated by basic lead acetate and ammonia. The washed precipitate is treated with boiling alcohol, filtered hot, the filtrate treated with a few drops of soda solution, and evaporated to dryness. The dry residue is extracted with absolute alcohol, filtered, and an excess of ether added. The amorphous or, after a longer time, crystalline, precipitate consisting of the alkali salts of the biliary acids is used in performing PETTENKOFER'S test.

Bile-pigments occur in the urine in different forms of icterus. A urine containing bile-pigments is always abnormally colored—yellow, yellowish brown, deep brown, greenish yellow, greenish brown, or nearly pure green. On shaking it froths, and the bubbles are yellow or yellowish green in color. As a rule icteric urine is somewhat cloudy, and the sediment is frequently, especially when it contains epithelium-cells, rather strongly colored by the bile-pigments.

Detection of Bile-coloring Matters in Urine. Many tests have been proposed for the detection of these substances. Ordinarily we obtain the best results with the following three tests:

GMELIN'S *test* may be applied directly to the urine; but it is better to use ROSENBACH'S modification. Filter the urine through a very small filter, which becomes deeply colored from the retained epithelium-cells and bodies of that nature. After the liquid has entirely passed through apply to the inside of the filter a drop of nitric acid which contains only very little nitrous acid. A pale-yellow spot will be formed which is surrounded by colored rings which appear yellowish red, violet, blue, and green from within outward. This modification is very delicate, and it is hardly possible to mistake indican and other coloring-matters for the bile-pigments. Several other modifications of GMELIN'S direct test, e.g., with concentrated sulphuric acid and nitrate, etc., have been proposed, but they are neither simpler nor more delicate than ROSENBACH'S modification.

HUPPERT'S *Reaction.* In a dark-colored urine or one rich in indican good results are not always obtained with GMELIN'S test. In such cases, as also in urines containing blood-coloring matters at the same time, the urine is treated with lime-water, or first with some $CaCl_2$ solution,

[1] Cited from Huppert-Neubauer, Harn-Analyse, 10. Aufl., 229.

and then with a solution of sodium or ammonium carbonate. The precipitate which contains the bile-coloring matter is filtered, washed, dissolved in alcohol which contains 5 cc. of concentrated hydrochloric acid in 100 cc. (I. MUNK), and heated to boiling, when the solution becomes green or bluish green. According to NAKAYAMA [1] this reaction is more delicate on using a mixture of ferric chloride, acid, and alcohol.

HAMMARSTEN'S *Reaction.* For ordinary cases it is sufficient to add a few drops of urine to about 2–3 cc. of the reagent (see page 432), when the mixture immediately after shaking turns a beautiful green or bluish green, which color remains for several days. In the presence of only very small quantities of bile-pigments, especially when blood or other pigments are simultaneously present, pour about 10 cc. of the acid or nearly neutral (not alkaline) urine into the tube of a small centrifugal machine and add $BaCl_2$ solution and centrifuge for about one minute. The liquid is decanted and the sediment stirred with about 1 cc. of the reagent and centrifuged again. A beautiful green solution is obtained which may be changed, by the addition of increased quantities of the acid mixture, to blue, violet, red, and reddish yellow. The green color may be obtained in the presence of 1 part bile-pigment in 500,000–1,000,000 parts urine. In the presence of large amounts of other pigments calcium chloride is better suited than barium chloride.

BOUMA [2] has suggested the use of alcohol containing ferric chloride and hydrochloric acid instead of the above-mentioned acid mixture. He has also worked out a colorimetric method of quantitative estimation of bilirubin in urine by means of this reagent.

As above indicated, we have a great many other tests besides these given above. A very complete summary of these tests and the literature thereof can be found in the work of OBERMAYER and POPPER.

For ordinary purposes the above-mentioned tests are sufficiently delicate, and according to HAMMARSTEN it is not advisable, as also in the case of the detection of proteid, sugar, etc., to increase the delicacy of a test so that it shows the presence of the traces of the questionable substance in normal urine. If in certain cases a greater delicacy is required than is obtained with the above tests, then we must recommend the flotation test of OBERMEYER and POPPER [3] with iodine and salt.

MEDICINAL COLORING-MATTERS produced from santonin, rhubarb, senna, etc., may give an abnormal color to the urine and may be mistaken for bile-pigments, or, in alkaline urines, perhaps for blood-coloring matters. If hydrochloric acid is added to such a urine, it becomes yellow or pale yellow, while on the addition of an excess of alkali it takes on a more or less beautiful red color.

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[1] Munk, Arch. f. (Anat. u.) Physiol., 1898; Nakayama, Zeitschr. f. physiol. Chem., **26**.

[2] Deutsch. med. Wochenschr., 1902 and 1904

[3] Wien. med. Wochenschr., **21**.

Sugar in Urine.

The occurrence of traces of glucose in the urine of perfectly healthy persons has been, as above stated (page 749), quite positively proven. If sugar appears in the urine in constant and especially in large quantities, it must be considered as an abnormal constituent. In a previous chapter several of the principal causes of glycosuria in man and animals were mentioned, and the reader is referred to Chapters VII and VIII for the essential facts in regard to the appearance of sugar in the urine.

In man the appearance of glucose in the urine has been observed under various pathological conditions, such as lesions of the brain and especially of the medulla oblongata, abnormal circulation in the abdomen, diseases of the heart, lungs and liver, cholera, and many other diseases. The continued presence of sugar in human urine, sometimes in very considerable quantities, occurs in DIABETES MELLITUS. In this disease there may be elimination of 1 kilogram or even more of glucose per day. In the beginning of the disease, when the quantity of sugar is still very small, the urine often does not appear abnormal. In the more developed, typical cases the quantity of urine voided increases considerably, to 3–6–10 liters per day. The percentage of the physiological constituents is as a rule very low, while their absolute daily quantity is increased. The urine is pale, but of a high specific gravity, 1.030–1.040 or even higher. The high specific gravity depends upon the quantity of sugar present, which varies in different cases, but may reach 10 per cent. The urine is therefore characterized in typical cases of diabetes by the very large quantity voided, by the pale color and high specific gravity, and by its containing sugar.

That the urine after the introduction into the system of certain medicinal agents or poisonous bodies contains reducing substances, conjugated glucuronic acids, which may be mistaken for sugar, has already been mentioned.

Glucose in urine. The properties and reactions of this sugar have been considered in a previous chapter, and it remains but to mention the methods for the detection and quantitative determination of glucose in the urine.

The *detection of sugar* in the urine is ordinarily, in the presence of not too small quantities, a very simple task. The presence of only very small quantities may make its detection sometimes very difficult and laborious. A urine containing proteid must first have the proteid removed by coagulation with acetic acid and heat before it can be tested for sugar.

The tests which are most frequently employed and are especially recommended are as follows:

TROMMER'S *Test.* In a typical diabetic urine or one rich in sugar this test succeeds well, and it may be performed in the manner suggested on

page 214. This test may lead to very great mistakes in urine poor in sugar, especially when they have at the same time normal or increased amounts of physiological constituents, and therefore it cannot be recommended to physicians or to persons inexperienced in such work. Normal urine contains reducing substances, such as uric acid, creatinine, and others, and therefore a reduction takes place in all urines on using this test. A separation of copper suboxide does not generally occur, but still if one varies the proportion of the alkali to the copper sulphate and boils, there takes place an actual separation of suboxide in normal urines, or a peculiar yellowish red liquid due to finely divided cuprous hydroxide. This occurs especially on the addition of much alkali or too much copper sulphate, and by careless manipulation the inexperienced worker may therefore sometimes obtain apparently positive results in a normal urine. On the other hand, as the urine contains substances such as creatinine and ammonia (from the urea), which in the presence of only a little sugar may keep the copper suboxide in solution, the investigator may easily overlook small quantities of sugar that may be present.

The delicacy of TROMMER'S test can be increased by the suggestion made by WORM-MÜLLER.[1] As by this rather complicated and tedious method small amounts of sugar cannot be detected in certain urines, and also as special urines from healthy persons readily give inconclusive results, and finally as SCHÖNDORFF has shown in numerous cases that the physiological sugar content of the urine responds to this test in perfectly healthy persons because of its extreme delicacy, it does not seem advisable in HAMMARSTEN'S opinion to recommend this test to the physician. BANG and BOHMANSSON[2] have recently also shown its unreliability.

ALMÉN'S *bismuth test*, which has been incorrectly called NYLANDER'S test, is performed with the alkaline-bismuth solution prepared as described on page 214. For each test 10 cc. of urine are taken and treated with 1 cc. of the bismuth solution and boiled for a few minutes. In the presence of sugar the urine becomes dark yellow or yellowish brown; then it grows darker, cloudy, dark brown, or nearly black, and non-transparent. After a longer or shorter time a black deposit appears, the supernatant liquid gradually clears, but still remains colored. In the presence of only very little sugar the test does not become black or dark brown, but simply deeper colored, and not until after some time is there seen on the upper layer of the phosphate precipitate a dark or black layer (of bismuth?). In the presence of much sugar a larger amount of the reagent may be used without disadvantage. In a urine poor in sugar only 1 cc. of the reagent for every 10 cc. of the urine must be employed.

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[1] In regard to this test see Pflüger, Pflüger's Arch., **105** and **106**; Hammarsten, *ibid.*, **116**, and Zeitschr. f. physiol. Chem., **50**.

[2] Schöndorff, Pflüger's Arch., **121**; Bohmansson, Bioch. Zeitschr., **19**.

Small amounts of proteid may retard this reaction and reduce the delicacy of the test. Large quantities of proteid may, however, give rise to an error by forming bismuth sulphide, and therefore it must always be first removed. The assertion of BECHHOLD that mercury compounds in the urine disturb the test has not been substantiated by ZEIDLITZ on properly performing the test, and recently REHFUSS and HAWK [1] came to the same conclusion. Those sources of error which in TROMMER'S test are caused by the presence of uric acid and creatinine are removed by using this test. The bismuth test is, moreover, readily performed, and on this account is to be recommended to the physician.

The bumping and ejection of the fluid can be readily prevented by heating over a very small flame after the test has been brought to a boil, and by gently shaking the contents of the not too narrow test-tube. The recommendation of heating for a longer time in the water-bath, fifteen minutes or more, is to be discarded, as the delicacy of the test is thereby so much increased that it gives a reaction with a physiological sugar content of 0.02 per cent.

When the amount of sugar in the urine is not less than 0.1 per cent a positive reaction is obtained if the test is boiled for 2–3 minutes and then allowed to stand quietly for 5 minutes. The phosphate precipitate is then black or nearly black. In detecting smaller quantities of sugar —0.05 per cent, the test as a rule must be boiled longer—about 5 minutes.

The value of this test lies in the fact that it positively detects small quantities of sugar—0.1 per cent or somewhat less, and that when the urine gives negative results we can consider it free from sugar in a clinical sense. Like TROMMER'S test it is a reduction test, and shows also certain other reducing bodies besides the sugar. These bodies are certain conjugated glucuronic acids which may appear in the urine. After the use of certain therapeutic agents, such as rhubarb, senna, antipyrine, salol, turpentine and others, the bismuth test gives positive results. From this it follows that we should never be satisfied with this test alone, especially when the reduction is not very great.

According to BOHMANSSON and BANG this test is perfectly reliable if about 20 cc. of the urine is treated with 5 cc. of 25 per cent HCl and 2 grams blood-charcoal (a teaspoonful) added and shaken every once in a while during five minutes and then filtered. The filtrate on neutralization with caustic soda is used for the ALMÉN test. The disturbing reducing substances are removed by the animal charcoal, but the sugar is not.

According to ANDERSEN [2] this procedure cannot be used in the quan-

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[1] Bechhold, Zeitschr. f. physiol. Chem., **46**; Zeidlitz, Upsala Läkaref. Förh. (N. F.), **11** (Hammarsten Festschr.); Rehfuss and Hawk, Journ. of biol. Chem., **7**.

[2] Bohmansson and Bang, Bioch. Zeitschr., **19**, and Zeitschr. f. physiol. Chem., **63**; Andersen, Bioch. Zeitschr., **37**.

titative estimation of sugar as a part of the sugar is retained by the use of hydrochloric acid and blood-charcoal. According to ANDERSEN the pigments and the disturbing substances can be removed by percipitation with mercuric nitrate. It can be more simply done by treating 40 cc. of the urine with 10 cc. acetic acid of 50 per cent strength and 4 grams blood-charcoal, shaking as above described and filtering. In the presence of acetic acid no sugar is taken up by the charcoal and as this simple method can be used for the quantitative estimation it can therefore be used in the qualitative tests for sugar.

Fermentation Test. On using this test the process must vary according as the bismuth test shows small or large quantities of sugar. If a rather strong reduction is obtained, the urine may be treated with yeast and the presence of sugar determined by the generation of carbon dioxide. In this case the acid urine, or that faintly acidified with a little tartaric acid is treated with compressed yeast, or yeast which has previously been washed by decantation with water. Pour this urine to which the yeast has been added into a SCHRÖTTER'S gas burette or a LOHNSTEIN'S saccharimeter (see below). As the fermentation proceeds, the carbon dioxide collects in the upper part of the tube, while a corresponding quantity of liquid is expelled below. As a control in this case two similar tests must be made, one with normal urine and yeast to learn the quantity of gas usually developed, and the other with a sugar solution and yeast to determine the activity of the yeast. According to VICTOROW [1] the fermentation is complete after six hours at a temperature of 34–36° C.

If, on the contrary, only a faint reduction with the bismuth test is found, no positive conclusion can be drawn from the absence of any carbon dioxide or the appearance of a very insignificant quantity. The urine absorbs considerable amounts of carbon dioxide, and in the presence of only small amounts of sugar the fermentation test as above performed may lead to negative or inaccurate results. In this case proceed in the following way: Treat the acid urine, or urine which has been faintly acidified with tartaric acid, with yeast whose activity has been tested by a special test on a sugar solution, and allow it to stand six to twelve hours at about 34–36° C. Then test again with the bismuth test, and if the reaction now gives negative results, then sugar was previously present. But if the reaction continues to give positive results, then it shows, if the yeast is active, the presence of other reducing, unfermentable substances.

In performing the fermentation test care should be taken that the urine be acid before as well as after fermentation. If the reaction becomes

[1] Pflüger's Arch., 118.

alkaline during fermentation (alkaline fermentation), then the test must be discarded. The vessel must be perfectly clean and strongly heated before use. To make sure the urine may be boiled before fermentation.[1]

If a good polariscope is at hand it must not be forgotten to control the results of the fermentation by determining the rotation before and after fermentation. The phenylhydrazine test also, in many otherwise doubtful cases, gives good service in testing urines for sugar.

Phenylhydrazine Test. Can be performed in the following manner: 20–25 cc. urine in a test-tube or in a beaker covered with a watch-glass are treated with 1 gram phenylhydrazine hydrochloride and 2 grams sodium acetate, and after solution of the salts it is warmed on the water-bath for three-quarters of an hour. In the presence of sugar even during the warming, a precipitate occurs, or in the presence of only a little sugar, at least after the gradual cooling, a yellow, crystalline precipitate forms. If the precipitate is very slight, it can be collected to advantage by means of a centrifuge and investigated by aid of the microscope. One finds at least a few phenylglucosazone crystals in the sediment while the appearance of smaller or larger yellow platelets or strongly refractive, brown globules is not indicative of sugar. In the presence of large amounts of sugar in the urine a large quantity of the yellow needles of phenylglucosazone or a mass of them are obtained.

This reaction is very reliable, and by it the presence of 0.03 per cent sugar can be detected (ROSENFELD, GEYER[2]). In doubtful cases it is necessary to investigate the nature of the precipitate. For this purpose dissolve a large quantity of the crystals in hot alcohol, treat the filtrate with water, and boil off the alcohol. Still better, the precipitate is dissolved, according to NEUBERG, in some pyridine, and again precipitated as crystals by the addition of benzene, ligroin, or ether. If the characteristic yellow crystalline needles, whose melting-point (204–205° C.) may also be determined, are now obtained, then this test is decisive for the presence of sugar. It must not be forgotten that fructose gives the same osazone as glucose, and that a further investigation is necessary in certain cases, and also that the impure crystals of phenylglucosazone have a much lower melting-point than the pure ones.

The following modification by A. NEUMANN is simple, practical, and at the same time sufficiently delicate. 5 cc. of the urine are treated with 2 cc. of acetic acid (30-per cent) saturated with sodium acetate, 2 drops of pure phenylhydrazine

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[1] On the performance of the fermentation test and certain sources of error, see Salkowski, Berlin. klin. Wochenschr., 1905 (Ewald-Festnummer), and Pflüger, Pflüger's Arch., **105** and **111**.

[2] Rosenfeld, Deutsch. med. Wochenschr., 1888; Geyer, cited from Roos, Zeitschr. f. physiol. Chem., **15**.

added, and the mixture boiled in a test-tube until it measures 3 cc. After quickly cooling warm again and then allow it to cool slowly. After 5–10 minutes beautifully formed crystals are obtained even in the presence of only 0.02 per cent sugar. According to the experience of HAMMARSTEN this modification, even in the presence of 0.1 per cent sugar in concentrated urines, does not always give a positive reaction. SALKOWSKI [1] has suggested an even more simple method.

The value of the phenylhydrazine test has been considerably debated, and the objection has been made that glucuronic acids also give a similar precipitate. A confounding with glucuronic acid is, according to HIRSCHL, not to be apprehended when the test is heated in the water-bath for a long time (one hour). KISTERMANN found this precaution insufficient, and Roos states that the phenylhydrazine test always gives a positive result with human urine, which coincides with E. HOLMGREN'S [2] and HAMMERSTEN'S experience. This test only shows a non-physiological quantity of sugar when a rather abundant crystallization is obtained from a small quantity of urine (about 5–10 cc.) Too great a delicacy of this test is not to be recommended.

RUBNER'S *test* is performed as follows: The urine is precipitated with an excess of a concentrated lead-acetate solution and the filtrate carefully treated with enough ammonia to produce a flocculent precipitate. It is then heated to boiling, when the precipitate becomes flesh-colored or pink in the presence of sugar.

Polarization. This test is of great value, especially as in many cases it quickly differentiates between glucose and other reducing, sometimes levogyrate, substances, such as the conjugated glucuronic acids. In the presence of only very little sugar the value of this test depends on the delicacy of the instrument and the dexterity of the observer. As a urine which shows no rotation or is actually faintly levorotatory, may contain 0.2 per cent glucose or perhaps even more, this test must be combined with the fermentation test if we are seeking very small amounts of sugar. The sugar in these cases can be detected only by the use of a very accurate and delicate instrument. This method is in many cases not serviceable for the physician. If the urine is to be clarified and partly decolorized by precipitation with lead acetate, it must be done in acid solution with acetic acid.[3]

In the isolation of sugar and carbohydrates from the urine the benzoic-acid esters may be prepared according to BAUMANN'S method. The urine is made alkaline with caustic soda to precipitate the earthy phosphates, the filtrate treated with 10 cc. of benzoyl chloride and 120 cc. of 10 per cent caustic soda solution for every 100 cc. of the filtrate (REINBOLD [4]), and shaken until the odor of benzoyl

[1] Neumann, Arch. f. (Anat. u.) Physiol., 1899, Suppl. See also Margulies, Berlin. klin. Wochenschr., 1900; Salkowski, Arbeiter aus dem pathol. Inst., Berlin, 1906.

[2] Hirschl, Zeitschr. f. physiol. Chem., 14; Kistermann, Deutsch. Arch. f. klin. Med., 50; Roos, l. c.; Holmgren, Maly's Jahresber., 27.

[3] See Grossmann, Bioch. Zeitschr., 1.

[4] Pflüger's Arch., 91.

chloride has disappeared. After standing sufficiently long the ester is collected, finely divided, and saponified with an alcoholic solution of sodium ethylate in the cold according to BAISCH'S method,[1] and the various carbohydrates separated according to his suggestion.

If small quantities of sugar are to be isolated from the urine, precipitate the urine first with sugar of lead, filter, precipitate the filtrate with ammoniacal basic lead acetate, wash this precipitate with water, decompose it with H_2S when suspended in water and use the filtrate for the special tests. SCHÖNDORFF[2] has suggested a method for the detection and estimation of very small amounts of sugar based upon the work of PATEIN and DUFAU. This method depends upon precipitating the nitrogenous substances with mercuric nitrate.

To the physician, who naturally wants simple and quick methods, the bismuth test is especially to be recommended. If this test gives negative results, the urine is to be considered as free from sugar in a clinical sense. If it gives positive results, the presence of sugar must be controlled by other tests, especially by the fermentation test.

Other tests for sugar, as, for example, the reaction with orthonitrophenylpropiolic acid, picric acid, diazobenzene-sulphonic acid, are superfluous. The reaction with α-naphthol, which is a reaction for carbohydrates in general, for glucuronic acid and mucin, may, because of its extreme delicacy, give rise to mistakes, and is therefore not to be recommenced to physicians. Normal urines give this test, and if the strongly diluted urine gives the reaction the presence of great quantities of carbohydrates may be suspected. In these cases more positive results are obtained by using other tests. This test requires great cleanliness, and it has the inconvenience that sufficiently pure sulphuric acid is not always readily procurable. Several investigators, such as V. UDRÁNSKY, LUTHER, ROOS and TREUPEL,[3] have investigated this test in regard to its applicability as an approximate test for carbohydrates in the urine.

Quantitative Determination of Sugar in the Urine. The quantity of sugar can be determined by *titration*, by *fermentation* of the sugar, by *polarization*, and also in other ways.

The titration methods are based upon the property of the sugar to reduce metallic oxides in alkaline solutions. As the titration liquids (cupric oxide solution in the FEHLING-SOXHLET, PAVY, BANG, BERTRAND methods and mercuric oxide in KNAPP'S method) are also reduced by other urinary constituents, these reduction methods always give too high results. When large quantities of sugar are present, as in typical diabetic urine, which generally contains a lower percentage of normal reducing constituents, this is indeed of little account; but when small quantities of sugar are present in an otherwise normal urine, the mistake may, on the contrary, be important, as the reducing power of normal urine may correspond to 5 p. m. glucose (see page 749). In such cases the titration procedure must be employed in connection with the fermentation method, which will be described later.

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[1] Zeitschr. f. physiol. Chem., **19**.

[2] Pflüger's Arch., **121**, which cites the work of Patein and Dufau.

[3] See Roos and Treupel, Zeitschr. f. physiol. Chem., **15** and **16**.

Of the titration methods with copper solutions the method suggested by BANG is the simplest, and at the same time seems to be more reliable than any of the others. For this reason we will describe only this method and refer to the original works and to HOPPE-SEYLER-THIERFELDER, Handbuch der Chem. Analyses, 1909, for description of the titration of FEHLING'S solution according to SOXHLET [1] and to the titration according to PAVY and KUMAGAWA-SUTO.[2]

BANG'S *First Method.*[3] The principle of this method is that when urine is boiled with an excess of a solution of potassium carbonate, potassium thiocyanate and copper sulphate, copper thiocyanide is formed, and this remains in solution as a colorless compound. The excess of cupric oxide remaining is determined by titration with a solution of hydroxylamine until the blue color disappears. The quantity of sugar is calculated from the quantity of hydroxylamine used.

The following solutions are necessary: (*a*) A copper salt solution containing 25 grams cupric sulphate in 2 liters, and (*b*) a solution containing 6.55 grams hydroxylamine sulphate in 2 liters.

The copper solution is prepared in the following manner: Dissolve 100 grams potassium bicarbonate in 1300 cc. water in a 2-liter graduated flask, and if necessary warm to 50–60° C. After complete solution of the bicarbonate, add 400 grams potassium thiocyanate and 500 grams potassium carbonate. To this solution, which must have the temperature of the room, add very slowly 150 cc. of a copper sulphate solution, which contains 166.67 grams copper sulphate ($CuSO_4+5H_2O$) per liter, then add water up to 2 liters. This solution unfortunately does not keep indefinitely, still, according to ANDERSEN, it can be kept in the dark up to 3 months and its strength controlled by titration with the hydroxylamine solution. The hydroxylamine solution is prepared by dissolving 200 grams potassium thiocyanate in about 1500 cc. water in a 2-liter graduated flask and adding a solution of 6.55 grams hydroxylamine sulphate in water; then add water to the 2-liter mark. This solution, on the contrary keeps, but it must be kept in a dark-colored bottle. Equal volumes of each of these two solutions should exactly correspond to each other, and this can be determined by titrating at ordinary temperature 50 cc. of the copper solution (plus 10 cc. water) with the hydroxylamine solution.

The presence of proteid does not interfere with the reaction, and it is not necessary to remove the proteid. The urine for titration should not contain more than 0.6 per cent sugar. If the amount is lower, then 10 cc. of urine is used directly; if it is higher, then the urine is correspondingly diluted and of this diluted urine we also make use of 10 cc. in the titration. The quantities of sugar given in the table below vary between 0.9 and 60 milligrams in 10 cc.

Performance of the Determination. 10 cc. of the sugar fluid are placed in a glass flask and treated with 50 cc. of the copper solution. This is heated on a wire-gauze to boiling, boiled for three minutes, cooled quickly with water to the temperature of the room and then the hydrox-

[1] Journ. f. prakt. Chem., (N. F.), **21**.

[2] Pavy, The Physiology of the Carbohydrates, London, 1894; Kumagawa and Suto, Salkowski's Festschr., 1904; Sahli, Deutsch. med. Wochenschr., 1905.

[3] Bang, Bioch. Zeitschr., **2**, 11, **32**, and **38**. See also Funk, Zeitschr. f. physiol. Chem., **56** and **69**; Jessen-Hansen, Bioch. Zeitschr., 10 and Andersen, *ibid.*, **15** and **26**.

ylamine solution allowed to flow in from a burette until the blue color disappears and the solution is colorless, or, in urine poor in sugar, is yellow. The sugar in milligrams is directly obtained from the amount of hydroxylamine solution used by referring to the following reduction table:[1]

Hydroxylamine solution used.	Milligrams sugar.	Hydroxylamine solution used.	Milligrams sugar.	Hydroxylamine solution used.	Milligrams sugar.	Hydroxylamine. solution used.	Milligrams sugar.
0.75	60.0	13.00	39.0	25.50	23.5	38.00	10.4
1.00	59.4	13.50	38.3	26.00	22.9	38.50	9.9
1.50	58.4	14.00	37.7	26.50	22.3	39.00	9.4
2.00	57.3	14.50	37.1	27.00	21.8	39.50	9.0
2.50	56.2	15.00	36.4	27.50	21.2	40.00	8.5
3.00	55.0	15.50	35.8	28.00	20.7	40.50	8.1
3.50	54.3	16.00	35.1	28.50	20.1	41.00	7.6
4.00	53.4	16.50	34.5	29.00	19.6	41.50	7.2
4.50	52.6	17.00	33.9	29.50	19.1	42.00	6.7
5.00	51.6	17.50	33.3	30.00	18.6	42.50	6.3
5.50	50.7	18.00	32.6	30.50	18.0	43.00	5.8
6.00	49.8	18.50	32.0	31.00	17.5	43.50	5.4
6.50	48.9	19.00	31.4	31.50	17.0	44.00	4.9
7.00	48.0	19.50	30.8	32.00	16.5	44.50	4.5
7.50	47.2	20.00	30.2	32.50	15.9	45.00	4.1
8.00	46.3	20.50	29.6	33.00	15.4	45.50	3.7
8.50	45.5	21.00	29.0	33.50	14.9	46.00	3.3
9.00	44.7	21.50	28.3	34.00	14.4	46.50	2.9
9.50	44.0	22.00	27.7	34.50	13.9	47.00	2.5
10.00	43.3	22.50	27.1	35.00	13.4	47.50	2.1
10.50	42.5	23.00	26.5	35.50	12.9	48.00	1.7
11.00	41.8	23.50	25.8	36.00	12.4	48.50	1.3
11.50	41.1	24.00	25.2	36.50	11.9	49.00	0.9
12.00	40.4	24.50	24.6	37.00	11.4		
12.50	39.7	25.00	24.1	37.50	10.9		

For every $\frac{1}{10}$ cc. hydroxylamine solution used more than given in the table between 49.00–15.00, subtract 0.1 milligram from the corresponding sugar value and 0.2 milligram between 15.00–1.0.

The yellow color of the urine may be somewhat disturbing for the end reaction so that with little experience an error of 0.5 cc. hydroxylamine solution (= about 0.5 milligram sugar) may occur. In order to decolorize the urine we can precipitate, according to ANDERSEN, with mercuric nitrate, when the greatest part of the disturbing reducing substances are removed, and then the excess of mercury removed by caustic soda and shaking with zinc. Still simpler is the suggestion mentioned on page 805 with blood-charcoal after acidification with acetic acid.

BANG[2] decolorizes by the addition of 2 cc. alcohol of 95–97 per cent and a teaspoonful of blood-charcoal to 18 cc. urine, shaking and filtering immediately. By this means 50 per cent of the other reducing substances

[1] This table is given with the permission of the publisher, Julius Springer, Berlin, where it can be obtained at a low cost.

[2] Andersen, Bioch. Zeitschr., 15 and 87; Bang, *ibid.*, 88.

are removed. If an acidified urine is used for the titration then the urine is added to the copper solution and not the reverse.

BANG'S *Second Method.* As the reagents necessary for the preceding titration are expensive, and as the copper solution only keeps for three months, and the preparation of the solutions requires great exactitude and is somewhat difficult, and as the method gives somewhat higher results than other reduction methods due to the high alkali and salt content of the solutions, BANG [1] has recently modified his original method. Instead of potassium thiocyanate he uses potassium chloride, which can also keep the cuprous oxide in solution as a colorless compound. Also the non-reduced cupric oxide remaining, as in the early method, is not determined, but the cuprous oxide formed in the reduction with the sugar is directly determined by titration. This is done by means of a N/100 (or N/10 or N/25) iodine solution, which in the alkaline liquid acts oxidizingly with the formation of cupric oxide, according to the formula: $CuCl+I+K_2CO_3=CuCO_3+KCl+KI$. Starch solution is used as indicator. As the potassium chloride can only hold small amounts of cuprous oxide in solution, and as the end-reaction with the blue iodine-starch cannot be determined with ease in the presence of large amounts of cupric oxide in solution, but can easily be done with the faintly blue coloration due to cupric oxide, by this method a maximum of 10 milligrams sugar can only be determined. On this account a urine rich in sugar must be diluted considerably before titration. It must also be remarked that the iodine does not only react with the cuprous oxide but also with other urinary constituents, and the importance of this method on titration with rich urines, poor in sugar, has not been sufficiently investigated. This method has given good results with pure sugar solutions and with blood; but as its use for the determination of sugar in the urine has not been sufficiently tested, we have only given the chief points of the method.

BERTRAND'S [2] *Titration Method* is more complicated than BANG'S method and does not seem to have any special advantages over this latter, at least in regard to the determination of sugar in the urine. A part of the cuprous oxide here also remains in solution and like the titration, according to FEHLING, the cuprous oxide sometimes settles only with difficulty. As this method seems to be used extensively we will give the principles of the method.

The method consists in boiling the sugar solution (sugar urine) with an excess of FEHLING'S solution. The cuprous oxide, freed from copper salt by decantation and washing (under special precautions), is dissolved by ferric sulphate in sulphuric acid, and the ferrous sulphate produced is determined by titration with potassium permanganate, standardized by oxalic acid. The equations of the reactions are as follows:

1. $Cu_2O+Fe_2(SO_4)_3+H_2SO_4=H_2O+2CuSO_4+2FeSO_4$
2. $10FeSO_4+2KMnO_4+8H_2SO_4=8H_2O+5Fe_2(SO_4)_3+2MnSO_4+K_2SO_4$.

2 Cu are equivalent to 2 Fe, and as these are equivalent to 1 mol. oxalic acid, then from the amount of oxalic acid (ammonium oxalate) used in the standardization of the potassium permanganate solution the quantity of copper separated as cuprous oxide can be readily calculated. The corresponding quantity of sugar may be found in a special table.

For exact determinations of sugar the method as suggested by ALLIHN and modified by PFLÜGER [3] is the best suited.

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[1] Bioch. Zeitschr., **49**.

[2] Bulletin de la Soc. chim., (3), **35**, (1906).

[3] Pflüger's Arch., **66**.

The TITRATION ACCORDING TO KNAPP depends on the fact that mercuric cyanide in alkaline solution is reduced to metallic mercury by glucose. The titration liquid should contain 10 grams of chemically pure dry mercuric cyanide and 100 cc. of caustic-soda solution of a specific gravity of 1.145 per liter. When the titration is performed as described below (according to WORM-MÜLLER and OTTO), 20 cc. of this solution should correspond to exactly 0.05 gram of glucose. If the process is carried out in other ways, the value of the solution is different.

In this titration also, the quantity of sugar in the urine should be between ½ and 1 per cent, and the extent of dilution necessary be determined by a preliminary test. To determine the end-reaction as described below, the test for the excess of mercury is made with sulphuretted hydrogen.

In performing the titration allow 20 cc. of KNAPP'S solution to flow into a flask and dilute with 80 cc. of water, or when the urine contains less than 0.5 per cent of sugar use only 40–60 cc. After this heat to boiling and allow the diluted urine to flow gradually into the hot solution, at first 2 cc., then 1 cc., then 0.5 cc., then 0.2 cc., and lastly 0.1 cc. After each addition let it boil ½ minute. When the end-reaction is approaching, the liquid begins to clarify and the mercury separates with the phosphates. The end-reaction is determined by taking a drop of the upper layer of the liquid into a capillary tube and then blowing it out on pure white filter-paper. The moist spot is first held over a bottle containing fuming hydrochloric acid and then over strong sulphuretted hydrogen. The presence of a minimum quantity of mercury salt in the liquid is shown by the spot becoming yellowish, which is best seen when it is compared with a second spot that has not been exposed to the gas. The end-reaction is still clearer when a small part of the liquid is filtered, acidified with acetic acid, and tested with sulphuretted hydrogen (OTTO)[1]. As the added quantity of urine contains 0.050 gram sugar the calculation of the percentage content in sugar, bearing in mind the extent of dilution, is very simple.

This titration, unlike the previous one, may be performed equally well by daylight and by artificial light. It is applicable even when the quantity of sugar in the urine is very small and that of the other urinary constituents is normal. It is more easily performed, and the titration liquids may be kept without decomposing for a long time (WORM-MÜLLER and his pupils[2]). There is diversity of opinion, nevertheless, among investigators on the value of this titration method.

ESTIMATION OF THE QUANTITY OF SUGAR BY FERMENTATION. This may be done in various ways: the simplest method, and one at the same time sufficiently exact for ordinary cases, is that of ROBERTS. This consists in determining the specific gravity of the urine before and after fermentation. In the fermentation of sugar, carbon dioxide and alcohol are formed as chief products, and the specific gravity is lowered, partly on account of the disappearance of the sugar and partly on account of the production of alcohol. ROBERTS found that a decrease of 0.001 in the specific gravity corresponded to 0.23 per cent sugar, and this has been substantiated since by several other investigators (WORM-MÜLLER and others). If the urine, for example, has a specific gravity of 1.030 before fermentation and 1.008 after, then the quantity of sugar contained therein was $22 \times 0.23 = 5.06$ per cent.

In performing this test the specific gravity must be taken at the same temperature before and after the fermentation. The urine must be faintly acid, and when necessary it should be acidified with a little hydro-

[1] Journal f. prakt. Chem., **26**.

[2] Pflüger's Arch., **16** and **23**.

chloric acid or sulphuric acid. The activity of the yeast must, when necessary, be controlled by a special test. Place 200 cc. of the urine in a 400 cc. flask, add a piece of compressed yeast the size of a pea, and subdivide the yeast through the liquid by shaking; close the flask with a stopper provided with a finely-drawn-out glass tube, and allow the test to stand at the temperature of the room or, still better, at 30–35° C. After twenty-four hours the fermentation is ordinarily ended, but this must be verified by the bismuth test. After complete fermentation filter through a dry filter, bring the filtrate to the proper temperature, and determine the specific gravity.

If the specific gravity be determined with a good pycnometer supplied with a thermometer and an expansion-tube, this method, when the quantity of sugar is not less than 0.4–0.5 per cent, gives, according to WORM-MÜLLER, very exact results, but this has been disputed by BUDDE.[1] For the physician the method in this form is not serviceable. Even when the specific gravity is determined by a delicate urinometer which can give the density to the fourth decimal, exact results are not obtained, because of the ordinary errors of the method (BUDDE); but the errors are usually smaller than those which occur in titrations made by unskilled hands.

When the quantity of sugar is less than 1.5 per cent, these methods cannot be used. Such small amounts cannot, as already mentioned, be determined by titration directly, because of the reducing power of normal urine. In such cases, it is better to first determine the reducing power of the urine by titration according to BANG or KNAPP, then ferment the urine with the addition of yeast and titrate again. The difference found between the two titrations calculated as sugar gives the true quantity of the latter.

The determination of the sugar by fermentation can be so performed that the loss in weight due to the CO_2 can be estimated, or the volume of the gas measured. For this last purpose LOHNSTEIN[2] has constructed a special fermentation saccharometer, and his "precision saccharometer" is to be recommended. Based upon LOHNSTEIN'S instrument, WAGNER[3] has constructed a "fermentation saccharo-manometer," which has certain advantages over LOHNSTEIN'S apparatus.

ESTIMATIONS OF SUGAR BY POLARIZATION. In this method the urine must be clear, not too deeply colored, and, above all, must not contain any other optically active substances besides glucose. The urine may contain several levorotatory substances such as proteids, β-oxybutyric acid, conjugated glucuronic acids, the so-called LEO'S sugar and less often cystine, all of which are unfermentable. The proteid is removed by coagulation, and the others are detected by the polariscope after complete fermentation. The fermentable fructose is detected in a special manner (see below), and the dextrorotatory milk-sugar differs from glucose in its not fermenting readily. By using a delicate instru-

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[1] Roberts, The Lancet, 1862; Worm-Müller, Pflüger's Arch., **33** and **37**; Budde, *ibid.*, **40**, and Zeitschr. f. physiol. Chem., **13**. See Lohnstein, Pflüger's Arch., **62**.

[2] Berlin. klin. Wochenschr., **35**, and Allg. med. Central-Ztg., 1899; Goldman, Chem. Centralbl., 1907, **1**, 1149.

[3] Münch. med. Wochensch., 1905.

ment and with sufficient practice very exact results can be obtained by this method. The value of this procedure consists in the rapidity with which the determination can be made. In using instruments specially constructed for clinical purposes the accuracy is less than with the less expensive fermentation test. Under such circumstances, and as the estimation by means of polarization can be performed with exactitude only by specially trained chemists, it is hardly worth while to give this method in detail, and the reader is referred to handbooks for hints in the use of the apparatus.

HASSELBACH and LINDHARD [1] have recently suggested a method for the quantitative estimation of sugar which is based on the decolorization of an alkaline safranin solution in the presence of sugar.

Fructose (levulose). Levogyrate urines containing sugar have been noted by several investigators, although the nature of the sugar was not well known to the earlier observers. In recent years several positively authentic cases of levulosuria have been described, and also cases of diabetes have been found where fructose exists in the urine besides glucose. Reports on this subject do not agree, however.[2]

Fructose may be detected as follows: The urine is levorotatory, and the levorotatory substance ferments with yeast. The urine gives the ordinary reduction tests and the ordinary phenylglucosazone. With methylphenylhydrazine it gives the characteristic fructose methylphenylosazone, and it also gives SELIWANOFF'S reaction on heating after the addition of an equal volume of hydrochloric acid and a little resorcin. With this test it must be remarked that too lengthy or too strong heating must not be applied, since other carbohydrates may also give the reaction (see page 218 and the works of ROSIN and UMBER [3]). In the presence of fructose a red coloration appears. After cooling it can be neutralized with soda and shaken out with amyl alcohol, (ROSIN) or with acetic ether (BORCHARDT). The amyl alcohol removes a red pigment which gives a band in the spectrum between *E* and *b* and on stronger concentration also a band in the blue at *F*. The acetic ether in the presence of fructose becomes yellow, and this is more characteristic according to BORCHARDT than ROSIN'S method, which has certain fallacies. The simultaneous presence of nitrites and indican disturbs the test, and in this case first remove the nitrous acid by boiling the urine, acidified with acetic acid or hydrochloric acid for one minute. In order to remove other disturbing pigments, MALFATTI suggests the oxidation of the urine with a little hydrochloric acid and potassium permanganate. JOLLES [4] has suggested a method for detecting fructose besides glucose by means of a diphenylamine solution.

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[1] Bioch. Zeitschr., **27**.

[2] See Borchardt, Zeitschr. f. physiol. Chem., **55** and **60**; W. Voit, *ibid.*, **58** and **61**; Adler, Pflüger's Arch., **139**.

[3] Umber, Salkowski's Festschrift, Berlin, 1904; Rosin, *ibid.*, and Zeitschr. f. physiol. Chem., **38**.

[4] Rosin, l. c.; Borchardt, l. c.; Malfatti, Zeitschr. f. physiol. Chem., **58**; Jolles and Mauthner, Chem. Centralbl., 1910, **1**, 483.

Maltose sometimes occurs in the urine according to LÉPINE and BOULUD. GEELMUYDEN,[1] who also held this view, now states that maltose does not occur in the urine.

Laiose is a substance named by HUPPERT and found by LEO [2] in diabetic urines in certain cases, and which he considers as a sugar. It is levogyrate, amorphous, and does not taste sweet, but rather sharp and salty. Laiose has a reducing action on metallic oxides, does not ferment, and gives a non-crystalline, yellowish-brown oil with phenylhydrazine. There is no positive proof as yet that this substance is a sugar.

Lactose. The appearance of lactose in the urine of pregnant women was first shown by the observations of DE SINETY and F. HOFMEISTER, and this has been substantiated by other investigators. After the ingestion of large quantities of milk-sugar some lactose may be found in the urine (see Chapter VIII on absorption). LANGSTEIN and STEINITZ have observed the passage of lactose and also of galactose [3] into the urine of nurslings with disease of the stomach. The passage of lactose into the urine is called lactosuria.

The positive detection of this sugar in the urine is difficult, because it is, like glucose, dextrogyrate, and also gives the usual reduction tests. If urine contains a dextrogyrate, non-fermentable sugar which reduces bismuth solutions, then it is very probable that it contains lactose. It must be remarked that the fermentation test for lactose is, according to the experience of LUSK and VOIT,[4] best performed by using pure cultivated yeast (*saccharomyces apiculatus*). This yeast only ferments the glucose while it does not decompose the milk-sugar. VOIT claims that if RUBNER'S test is performed without heating to boiling, but only to 80° C., the color becomes yellow or brown in the presence of lactose, instead of red. The most positive means for the detection of this sugar is to isolate the sugar from the urine. This may be done by the method suggested by F. HOFMEISTER.[5]

R. BAUER [6] detects galactose as well as lactose in the urine by oxidation with concentrated nitric acid, producing mucic acid.

Cammidge's reaction, which is recommended in the diagnosis of acute diseases of the pancreas, consists in that certain urines do not give the phenylhydrazine reaction directly, but only after boiling with an acid. The reason of this is not known and the reaction is partly due to cane-sugar, in part to pentoses or glucoronic acid and in part to mixtures of bodies.

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[1] Lépine and Boulud, Compt. Rend., 132; Geelmuyden, Zeitschr. f. klin, Med., 70.

[2] Virchow's Arch., 107.

[3] Hofmeister, Zeitschr. f. physiol. Chem., 1, which also contains the pertinent literature. See also Lemaire, *ibid.*, 21; Langstein and Steinitz, Hofmeister's Beiträge, 7.

[4] Carl Voit, Ueber Die Glycogenbildung nach Aufnahme verschiedener Zuckeraten, Zeitschr. f. Biologie, 28.

[5] Hofmeister, Zeitschr. f. physiol. Chem., 1, which also contains the pertinent literature.

[6] Zeitschr. f. Physiol. Chem., 51.

Pentoses. SALKOWSKI and JASTROWITZ first found in the urine of persons addicted to the morphine habit a variety of sugar which was a pentose and yielded an osazone which melted at 159° C. Since this several other cases of pentosuria have been observed, and according to KÜLZ and VOGEL and others small amounts of pentose also occur in the urine of diabetics, as also in the urine of dogs with pancreatic or phlorhizin diabetes.[1]

The pentose isolated by NEUBERG from the urine in chronic pentosuria was *d-l*-arabinose. LUZZATTO and KLERCKER studied cases of pentosuria and found *l*-arabinose. In alimentary pentosuria the *l*-arabinose of the plant food may be found in the urine. The appearance of pentoses in the urine after eating fruits and fruit-juices has been repeatedly observed by BLUMENTHAL and also by v. JAKSCH. According to COMINOTTI [2] pentoses habitually occur in human urine on a mixed diet.

A urine containing pentose reduces bismuth as well as copper solutions, although the reduction is not so rapid, but appears gradually. If only pentose is present, the urine does not ferment, but in the presence of glucose small amounts of pentose may also undergo fermentation. The preparation of the osazone serves in the detection of pentoses and when obtained from the urine it melts at 156–160° C. The phloroglucin or orcin tests can also be employed (see page 209). Of these the last is most preferable, especially as it excludes a confusion with the conjugated glucuronic acids.

The orcin test can be performed as follows: 5 cc. of the urine are mixed with an equal volume of HCl sp.gr. 1.19, a small amount of orcin added and the whole heated to boiling. As soon as a greenish cloudiness appears cool the mixture off and shake carefully with amyl alcohol. The amyl-alcohol solution is used in the spectroscopic examination. The precipitation of a bluish-green pigment is in itself significant.

BIAL [3] uses as reagent 30 per cent hydrochloric acid, which contains 1 gram of orcin and 25 drops of a ferric-chloride solution (62.9 per cent of the crystalline salt) in 500 cc. of the acid. 4.5 cc. of the reagent are heated to boiling and then a few drops (not more than 1 cc.) of the urine are added to the hot but not boiling liquid. In the presence of pentose the liquid turns a beautiful green. The usefulness of BIAL's reagent is questioned by several experimenters. The delicacy is too great and the possibility of confounding with other carbohydrates is not excluded. In regard to the numerous modifications of this test and to JOLLES' reaction we refer to page 209. The same for the quantitative estimation of pen-

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[1] In regard to the literature, see footnote 1, page 208. See also Blumenthal, "Die Pentosurie," Deutsche Klinik, 1902.

[2] Blumenthal, Deutsche Klinik, 1902; v. Jaksch, Centralbl. f. innere Medizin, 1906; Cominotti, Bioch. Zeitschr., 22.

[3] Deutsch. med. Wochenschr., 1903; see also footnote 4, page 209.

toses. JOLLES[1] considers the preparation of the osazone as a specially conclusive test and the distillation of the osazone with hydrochloric acid and testing the distillate with BIAL'S reagent.

ROSENBERGER[2] believes he has detected a *heptose* in the urine in a case of diabetes. According to him and to GEELMUYDEN[3] probably different varieties of sugar, which are not well known, can possibly occur in urine of diabetics.

Conjugated Glucuronic Acids. Certain conjugated glucuronic acids such as menthol- and turpentine-glucuronic acid may spontaneously decompose in the urine, and in this case they may readily lead to a confusion with pentoses. The urine should always be fresh as possible for these examinations.

A confusion of the glucuronic acids, which have a reducing power on copper or bismuth solutions, with glucose and fructose, can be prevented by the fermentation test. They may also be distinguished from glucose by their optical behavior, as the conjugated glucuronic acids are levogyrate. On boiling with an acid, dextrorotatory glucuronic acid is produced and the levorotation is changed to dextrorotation.

The conjugated glucuronic acids, like the pentoses, give the phloroglucin-hydrochloric-acid test. On the contrary they do not give the orcin test directly, but only after cleavage with the setting free of glucuronic acid. On using BIAL'S reagent no mistaking for pentoses occurs, although this statement requires further substantiation. The pentoses may also be isolated and identified by their osazones. Certain readily decomposable glucuronic acids can here give phenylhydrazine compounds. In order to detect glucuronic acid in the osazone precipitate, we can, as suggested by NEUBERG and SANEYOSHI[4] take a knife point (about 8 milligrams) of the precipitate, dissolve in 4 cc. strong hydrochloric acid, dilute with 4 cc. water, heat to boiling, add at least 0.1 gram naphthoresorcin, warm for ½ minute, allow to slowly cool to 50° and shake with benzene. In the presence of glucuronic acid the benzene solution is violet with an absorption in the yellowish-green.

The occurrence of conjugated glucuronic acids in the urine is shown when the urine does not give the orcin-hydrochloric-acid reaction directly, but only after boiling with the acid. The naphthoresorcin reaction, as suggested by TOLLENS, can also be used. To 5 cc. urine add 0.5 cc. of a 1 per cent alcoholic solution of naphthoresorcin and 5 cc. hydrochloric acid (sp.gr. 1.19), boil for one minute, allow to stand four minutes,

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[1] Jolles, Bioch. Zeitschr., **2**, Centralbl. f. inn. Med., 1907 and 1912, and Zeitschr. f. anal. Chem., **46**.

[2] Zeitschr. f. physiol. Chem., **49**.

[3] Rosenberger, Centralbl. f. inn. Med., **28**; Geelmuyden, Zeitschr. f. klin. Med., **58**, **62**, and **70**.

[4] Bioch. Zeitschr., **36**.

cool and shake with ether. In the presence of glucuronic acid the ether becomes violet or blue, and shows the absorption bands given on page 223. According to NEUBERG this test, which is not specific for glucuronic acid, is best performed with the naphthoresorcin in substance. This test is more conclusive, if, as suggested by NEUBERG and SCHEWKET,[1] the residue from an ethereal extract of the acidified urine is used.

The surest method is that suggested by MAYER and NEUBERG, which consists in precipitating the urine with basic lead acetate, decomposing the precipitate with H_2S, boiling with dilute sulphuric acid in order to split the conjugated acid, and then after neutralizing with soda, preparing the characteristic bromphenylhydrazine compound of glucuronic acid (see page 223) with *p*-bromphenylhydrazine hydrochloride and sodium acetate. HERVIEUX [2] has slightly modified this method. In regard to the quantitative estimation of glucuronic acid we must refer to the work of C. TOLLENS.[3]

Inosite seems to be a normal urinary constituent, although it occurs only in very small quantities (HOPPE-SEYLER, STARKENSTEIN [4]). In diabetes insipidus, as well as after excessive drinking of water, it occurs in large quantities in the urine because of a more abundant washing-out of the tissues.

For the detection of inosite we make use of the method given on page 581, with the modifications suggested by MEILLÈRE and STARKENSTEIN.

Acetone Bodies (acetone, acetoacetic acid, β-oxybutyric acid). These bodies, whose occurrence in the urine and formation in the organism have been the subject of numerous investigations, occur in the urine especially in diabetes mellitus, but also in many other diseases.[5] According to v. JAKSCH and others, acetone is a normal urinary constituent, though it may occur only in very small amounts (0.01 gram in twenty-four hours).

In regard to the origin of these bodies it was formerly considered that they were produced by an increased destruction of protein. One of the various reasons for this was the increase in the elimination of acetone

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[1] B. Tollens, Ber. d. d. chem. Gesellsch., **41**, 1788, and C. Tollens, Zeitschr. f. physiol. Chem., **56**; Neuberg, Bioch. Zeitschr., **24**; Neuberg and Schewket, *ibid.*, **44**; see also Mandel and Neuberg, *ibid.*, **18**.

[2] Mayer and Neuberg, Zeitschr. f. physiol. Chem., **29**; Hervieux, Compt. rend. soc. biol., **63**.

[3] Zeitschr. f. physiol. Chem., **61**.

[4] Starkenstein, Zeitschr. f. exp. Path. u. Therap., **5**, which contains the literature.

[5] In regard to the extensive literature on acetone bodies the reader is referred to Huppert-Neubauer, Harn-Analyse, 10. Aufl., and v. Noorden's Lehrb. d. Pathol. des Stoffwechsels. Berlin, 1906, and for recent work, Magnus-Levy, Die Azetonkörper, Ergbn. d. inn. Med. u. Kinderheilk., I.

and acetoacetic acid during inanition (v. JAKSCH, FR. MÜLLER [1]). This also stands in accord with the observations that a considerable increase in the quantity of acetone and acetoacetic acid eliminated is observed in such diseases as fevers, diabetes, digestive disturbances, mental diseases with abstinence and cachexia, where the body protein is largely destroyed. The formation of acetone bodies from protein is also indicated by the fact that acetone has been obtained as an oxidation product from gelatin and protein (BLUMENTHAL and NEUBERG, ORGLER [2]). The investigations of EMBDEN and collaborators are more conclusive. After EMBDEN and KALBERLAH showed that the liver is an organ where acetone is formed, EMBDEN, SALOMON and SCHMIDT [3] showed by experiments on extirpated livers, that butyric acid, oxybutyric acid, leucine, tyrosine and in fact those aromatic bodies which, like tyrosine, phenylalanine, phenyl-α-lactic acid and homogentisic acid contain a combustible benzene nucleus, are transformed, in the liver, into acetone. Research, which has been continued further by EMBDEN and his collaborators and substantiated by others, such as BAER, and BLUM, BORCHARDT and LANGE, NEUBAUER and GROSS, SCHMITZ and FR. SACHS [4] has shown that there can be no doubt that certain amino-acids, especially leucine, are strong acetone formers, and consequently that acetone can be formed from protein. Protamines and histones can also increase the acetone elimination (BORCHARDT) or, as we say, may have a "ketoplastic" action, and it is therefore possible that acetone can be formed from arginine with α-amino-valerianic acid as intermediary step (BORCHARDT and LANGE).

As we cannot deny the possibility of a formation of acetone from proteins, on the other hand we have observations which are inconsistent with the origin of the acetone bodies entirely from the proteins. Thus no parallelism exists between the acetone bodies and the nitrogen excretion in diabetics, and the fact, that in man no certain relation exists between the acetone elimination and the nitrogen and sulphur excretion, seems to show that the acetone bodies are not entirely derived from the proteins. In man the excretion of acetone does not increase with the rise in the

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[1] v. Jaksch, Ueber Acetonurie und Diaceturie. Berlin, 1885; Fr. Müller, Bericht über die Ergebnisse des an Cetti ausgeführten Hungerversuches. Berlin. klin. Wochenschr., 1887.

[2] Blumenthal and Neuberg, Deutsch. med. Wochenschr., 1901; Orgler, Hofmeister's Beiträge, 1.

[3] Hofmeister's Beiträge, 8.

[4] Embden, *ibid.*, 11, with Marx, Engel, Lattes and Michaud, *ibid.*, 11; Baer and Blum, Arch. f. exp. Path. u. Pharm., 55, 56, and 62; Borchardt, *ibid*, 53, with Lange, Hofmeister's Beiträge, 9; Neubauer and Gross, Zeitschr. f. physiol. Chem., 67; Schmitz, Bioch. Zeitschr., 28; Fr. Sachs, *ibid.*, 27.

quantity of protein, and an increase in the latter above the average causes a diminution in the elimination of acetone (ROSENFELD, HIRSCHFELD, FR. VOIT[1]).

The carbohydrates cannot be considered as material for the formation of acetone bodies. It is generally admitted that in man the exclusion of carbohydrates from the food or the diminution in their amount or their assimilation may lead to more or less increased elimination of acetone bodies. This behavior may occur in diabetes as well as in starvation and in the above-mentioned diseased conditions. The increased elimination of acetone with food lacking carbohydrates also occurs in healthy persons with a fatty diet but with a sufficient supply of calories in other ways (alimentary acetonuria). With an abundant supply of carbohydrates the elimination of acetone bodies may be greatly diminished or even stopped entirely. The carbohydrates therefore act "antiketoplastic," and a similar retarding action can be produced by certain other substances, such as glycerin (HIRSCHFELD), lactic acid and glutaric acid (BAER and BLUM) alanine and asparagin (FORSSNER, BORCHARDT and LANGE[2]). Certain bodies like glycerine, lactic acid, alanine, asparagin, which cause a sugar formation or increased elimination of sugar, act in the same way.

It must not be overlooked that the conditions are different in man and in other carnivora (GEELMUYDEN, FR. VOIT). In dogs the elimination of acetone bodies is not increased in starvation, but is reduced; it is augmented with increased quantities of meat, runs parallel with the nitrogen excretion, and is not diminished by carbohydrates (FR. VOIT[3]). In spite of this divergent behavior an unmistakable relation also exists in the dog between the elimination of acetone bodies and the carbohydrate metabolism, because in phlorhizin diabetes the *acidosis* occurs only after the glycogen has been consumed (MARUM[4]).

As the carbohydrates cannot be acetone-formers, then a second source only remains, namely, the fats. As proof of this there are certain cases of diabetes with strong elimination of acetone bodies (β-oxybutyric acid) where the quantity of protein transformed was too small to account for the acetone bodies (MAGNUS-LEVY). The free elimination of acetone bodies in starvation may also depend upon the fact that a great part of

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[1] Hirschfeld, Zeitschr. f. klin. Med., **28**; Geelmuyden, see Maly's Jahresber., **26**, and Zeitschr. f. physiol. Chem., **23** and **26**; Rosenfeld, Centralbl. f. innere Med., **16**; Voit, Deutsch. Arch. f. klin. Med., **66**.

[2] Borchardt and Lange, l. c.; Hofmeister's Beiträge, **9**; which also cites other works; Baer and Blum, *ibid.*, **10**; Forssner, Skand. Arch. f. Physiol., **25**.

[3] See footnote 1.

[4] Hofmeister's Beiträge, **10**.

the body fat is consumed, and in several cases a certain relation has been found between the fat consumed and the acetone bodies eliminated. Certain investigators (GEELMUYDEN, SCHWARZ, WALDVOGEL) have also observed an increase in the acetonuria on partaking of fatty food, and FORSSNER[1] has indeed found a certain parallelism between the acetone elimination and the fat taken up. For the present the fats are considered as the most important source of the acetone bodies.

The three acetone bodies occurring in the urine, as above stated, are acetone, acetoacetic acid and β-oxybutyric acid, and this last is considered as the mother-substance of the other two. If β-oxybutyric acid, $CH_3.CHOH.CH_2.COOH$, is introduced into the animal body, it is burnt if the quantity is not too great, while if in excess it passes into the urine as acetoacetic acid, $CH_3.CO.CH_2.COOH$. This acid can also be burnt, but if large quantities are introduced it appears in part in the urine and readily splits into acetone, $CH_3.CO.CH_3$, and CO_2. Acetone is in part burnt in the animal body, but a part is eliminated by the kidneys and especially by the lungs. We can imagine that the β-oxybutyric acid is a physiological metabolic product which normally is completely changed into acetoacetic acid and acetone, and in diabetes and especially with lack of carbohydrates is formed to an increased extent, or its combustion made more difficult, so that in the first place acetone and acetoacetic acid pass into the urine and in severe cases also β-oxybutyric acid (acidosis). In this connection it must be borne in mind that, because of the previously-mentioned (page 774) reversibility of the process, the direction may also be reversed, that is acetoacetic acid can also be changed into β-oxybutyric acid in the animal body and this has been proven by perfusion of livers (FRIEDMANN and MAASE) as well as in animals (DAKIN) and in diabetics (O. NEUBAUER)[2].

Since leucine in perfusion experiments with livers yields acetoacetic acid (EMBDEN and ENGEL) and also, as BAER and BLUM[3] found, that leucine and isovaleric acid increased the β-oxybutyric acid elimination in diabetics, it has been accepted that a formation of β-oxybutyric acid takes place from the leucine with isovaleric acid as an intermediary product:
(leucine $(CH_3)_2CH.CH_2.CH(NH_2).COOH \rightarrow (CH_3)_2CH.CH_2.COOH$, isovaleric acid).
Valine (α-amino-valeric acid $(CH_3)_2CH.CH(NH_2).COOH$) is, on the contrary, not an acetone former.

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[1] Magnus-Levy, Arch. f. exp. Path. u. Pharm., **42**; Geelmuyden, l. c., and Norsk, Magasin for Laegevidenskaben, 1900; see also Zeitschr. f. physiol. Chem., **41**; Schwarz, Deutsch. Arch. f. klin. Med., 1903; Waldvogel, Centralbl. f. inn. Med., **20**; Forssner, Skand. Arch. f. Physiol., **22** and **23**.

[2] Friedmann and Maase, Bioch. Zeitschr., **27**; Dakin, Journ. of biol. Chem., **8**; Neubauer, Maly's Jahresb., **40**, 849.

[3] Arch. f. exp. Path. u. Pharm., **55** and **56**; Embden and Engel, Hofmeister's Beiträge, **11**.

In regard to the formation of acetone bodies from fat it must be remarked that glycerin has an antiketoplastic action, and that the fatty acids can only be considered. As to the behavior of these in the formation of acetone, EMBDEN and MARX[1] have shown that only those normal fatty acids which contain an even number of carbon atoms are acetone formers, while those with an uneven number of carbon atoms are without action in this regard. This is true at least for the acids from *n*-decanoic acid to *n*-butyric acid, which latter is a strong acetone former. As in diabetics a greater number of oxybutyric acid molecules can be eliminated than corresponds to the number of fatty acid molecules decomposed, it seems as if more than one molecule of β-oxybutyric acid is produced from one molecule of fatty acid. We cannot therefore admit of a simple demolition of the fatty acids to butyric acid (by consecutive oxidation attacks in the β-position), but rather a destruction of the fatty acid molecules into several parts, and these take part in the formation of β-oxybutyric acid.

A synthetical formation of β-oxybutyric acid has been accomplished by GEELMUYDEN and others, but especially by MAGNUS-LEVY, starting with acetaldehyde, according to the hypothesis of SPIRO. It is also interesting that FRIEDMANN[2] has shown by perfusion experiments with livers that aldehyde ammonia, and to a greater extent aldol, are acetone formers. It must therefore be admitted that first a condensation of the aldehyde to aldol takes place, $CH_3.COH + CH_3.COH = CH_3.CH(OH).CH_2.COH$, and that β-oxybutryic acid, $CH_3.CH(OH).CH_2.COOH$, is formed from this by oxidation.

According to the above-mentioned perfusion experiments it must be admitted that the liver is important in the formation of acetone bodies, and EMBDEN and LATTES have found that the ability of the liver of the dog with pancreas diabetes or phloridzin diabetes to produce acetone is much greater than the liver of the normal animal. On the other hand, as shown by EMBDEN and MICHAUD,[3] in dogs and oxen the liver also has a strong destructive action upon acetoacetic acid. A similar action is also found in the kidneys, muscles and spleen of dogs and pigs. The destructive action of fresh organs is much stronger upon acetoacetic acid than upon acetone. They could not find any special cleavage products, and the above-mentioned, so-called demolition may therefore perhaps in part be a reformation of β-oxybutyric acid from the acetoacetic acid.

Acetone, C_3H_6O, dimethylketone, $CH_3.CO.CH_3$, is a thin, water-clear liquid, boiling at 56.3° and possessing a pleasant odor of fruit,

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[1] Hofmeister's Beiträge, **11**.

[2] Geelmuyden, Zeitschr. f. physiol. Chem., **23** and **26**; Magnus-Levy, Arch. f. exp. Path. u. Pharm., **42**; Friedmann, Hofmeister's Beiträge, **11**.

[3] Embden and Lattes, Hofmeister's Beiträge, **11**; Embden and Michaud, *ibid.*, **11**.

which in diabetes gives a pomaceous or fruit odor to the urine as well as the expired air. It is lighter than water, with which it mixes in all proportions, also with alcohol and ether. The most important reactions for acetone are the following:

LIEBEN'S *Iodoform Test.* When a watery solution of acetone is treated with alkali and then with some iodo-potassium-iodide solution and gently warmed, a yellow precipitate of iodoform is produced, which is known by its odor and by the appearance of the crystals (six-sided plates or stars) under the microscope. This reaction is very delicate, but it is not characteristic of acetone. GUNNING'S *modification of the iodoform test* consists in using an alcoholic solution of iodine and ammonia instead of the iodine dissolved in potassium iodide and alkali hydroxide. In this case, besides iodoform, a black precipitate of nitrogen iodide is formed, but this gradually disappears on standing, leaving the iodoform visible. This modification has the advantage that it does not give any iodoform with alcohol or aldehyde. On the other hand, it is not quite so delicate, but still it detects 0.01 milligram of acetone in 1 cc.

FROMMER'S [1] *Test.* This reagent is a 10 per cent alcoholic solution of salicylaldehyde. Add 1–2 cc. of this solution to 10 cc. of the solution (urine) and add to this mixture 1 gram KOH in substance, when a carmine-red color will be observed. If necessary warm to about 70° C. This reaction is just as delicate as the above.

REYNOLD'S *Mercuric-oxide Test* is based on the power of acetone to dissolve freshly precipitated HgO. A mercuric-chloride solution is precipitated by alcoholic caustic potash. To this add the liquid to be tested, shake well, and filter. In the presence of acetone the filtrate contains mercury, which may be detected by ammonium sulphide. This test has about the same delicacy as GUNNING'S test. Aldehydes also dissolve appreciable quantities of mercuric oxide.

LEGAL'S *Sodium Nitroprusside Test.* If an acetone solution is treated with a few drops of a freshly prepared sodium-nitroprusside solution and then with caustic-potash or soda solution, the liquid is colored ruby-red. Creatinine gives the same color; but if the mixture is saturated with acetic acid, the color becomes carmine or purplish red in the presence of acetone, but yellow and then gradually green and blue in the presence of creatinine. With this test paracresol responds with a reddish-yellow color, which becomes light pink when acidified with acetic acid and cannot be mistaken for acetone. ROTHERA [2] has suggested a modification which is more delicate by using ammonium salts and ammonia.

PENZOLDT'S *Indigo Test* depends on the fact that orthonitrobenzaldehyde in alkaline solution with acetone yields indigo. A warm saturated and

[1] Berl. klin. Wochenschr., 1905.

[2] Journ. of Physiol., 37.

then cooled solution of the aldehyde is treated with the liquid to be tested for acetone and next with caustic soda. In the presence of acetone the liquid first becomes yellow, then green, and lastly indigo separates; and this may be dissolved with a blue color by shaking with chloroform; 1.6 milligrams acetone can be detected by this test.

Acetoacetic Acid, $C_4H_6O_3$, acetylacetic acid, diacetic acid, $CH_3.CO.CH_2.COOH$, is a colorless, strongly acid liquid which mixes with water, alcohol, and ether in all proportions. On heating to boiling with water, and especially with acids, it decomposes into carbon dioxide and acetone, and therefore gives the above-mentioned reactions for acetone. It differs from acetone in that it gives a violet-red or brownish-red color with a dilute ferric-chloride solution. For the detection of this acid we make use of the following reactions which may be applied directly to the urine:

GERHARDT'S *Reaction.* Treat 10–15 cc. of the urine with ferric-chloride solution until it fails to give a precipitate filter, and add some more ferric chloride. In the presence of acetoacetic acid a wine-red color is obtained. The color becomes paler at the room temperature within twenty-four hours, but more quickly on boiling (differing from salicylic acid, phenol, sulphocyanides). A portion of the urine slightly acidified and boiled does not give this reaction on cooling, on account of the decomposition of the acetoacetic acid.

ARNOLD and LIPLIAWSKY'S *Reaction.* 6 cc. of a solution containing 1 gram of *p*-aminoacetophenone and 2 cc. of concentrated hydrochloric acid in 100 cc. of water are mixed with 3 cc. of a 1 per cent potassium-nitrite solution and then treated with an equal volume of urine. A few drops of concentrated ammonia are now added and violently shaken. A brick-red coloration is obtained. Then take 10 drops to 2 cc. of this mixture (according to the quantity of acetoacetic acid in the urine), add 15–20 cc. HCl of sp.gr. 1.19, 3 cc. of chloroform, and 2–4 drops of ferric-chloride solution and mix without shaking. In the presence of acetoacetic acid the chloroform is colored violet or blue (otherwise only yellowish or faintly red). This reaction is more delicate than the preceding test and reacts with 0.04 p.m. acetoacetic acid. Large amounts of acetone (but not the quantity occurring in urines) give this reaction according to ALLARD.[1]

BONDI and SCHWARZ'S[2] *Reaction.* 5 cc. of the urine is treated drop by drop with iodine-potassium iodide solution until the color is orange-red. Then warm gently and when the orange-red color has disappeared add the iodine solution again until the color remains permanent on warming. Then boil, when the irritating vapors of iodo-acetone will attack the eyes. Acetone does not give this reaction.

[1] Arnold, Wien. klin. Wochenschr., 1899, and Centralbl. f. innere Med., 1900; Lipliawsky, Deutsch. med. Wochenschr., 1901; Allard, Berl. klin. Wochenschr., 1901.

[2] Wien. klin. Wochenschr., 1906.

Detection of Acetone and Acetoacetic Acid in the Urine. Before testing for acetone test for acetoacetic acid; as this acid gradually decomposes on allowing the urine to stand, the specimen must be as fresh as possible. In the presence of acetoacetic acid the urine gives the above-mentioned tests. In testing for acetone in the presence of acetoacetic acid make the urine slightly alkaline and shake in a separatory funnel with ether free from alcohol and acetone. Remove the ether and shake it with water, which takes up the acetone, and test for acetone in the watery solution.

In the absence of acetoacetic acid the acetone may be tested for directly in the urine; this may be done by FROMMER'S test or LEGAL'S test. These tests, which are only approximate, are of value only when the urine contains a considerable amount of acetone.

For a more accurate test we distill at least 250 cc. of the urine faintly acidified with sulphuric acid, care being taken to have a good condensation. Most of the acetone is contained in the first 10–20 cc. of the distillate. A better result may be obtained by distilling a large quantity of urine until about $\frac{1}{10}$ has been distilled off, acidify the distillate with hydrochloric acid, redistill and repeat this several times, collecting the first portion of each distillation. The final distillate is used for the above reactions.[1] SALKOWSKI and BORCHARDT have called attention to the fact that in the distillation of an acidified urine containing sugar for the detection or estimation of acetone, a substance giving iodoform can be formed from the sugar if the distillation is carried too far. According to BORCHARDT [2] the urine must therefore first be diluted with water, or the concentration prevented by the addition of water dropwise during distillation.

The *quantitative estimation* of acetone (also that formed from the acetoacetic acid) is done by distilling the urine after the addition of acetic acid or a little sulphuric acid. The quantity of acetone in the distillate can be determined, according to the HUPPERT-MESSINGER method, by converting it into iodoform by means of potassium iodide and then titrating the quantity of iodine used in the formation of the iodoform. The precipitation of the acetone as *p*-nitrophenylhydrazone-acetone by means of *p*-nitrophenylhydrazine in acetic acid solution can also be used for determining the acetone in the distillate (v. EKENSTEIN and BLANKSMA and MÖLLER). In regard to these methods we refer to.[3] EMBDEN and SCHLIEP and FOLIN [4] have suggested methods for determining the quantity of acetone and acetoacetic acid separately. In regard to these estimations we must refer to the work of EMBDEN and SCHMITZ.[5]

β-Oxybutyric Acid, $C_4H_8O_3$, $=CH_3.CH(OH).CH_2.COOH$, ordinarily forms an odorless syrup, but may also be obtained as crystals. It is readily soluble in water, alcohol, and ether. It is levorotatory; $(\alpha)_D = -24.12°$

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[1] See also Salkowski, Pflüger's Arch., **56**.

[2] Hofmeister's Beiträge, 8.

[3] Hoppe-Seyler, Thierfelder, 8. Aufl., 617 and 618.

[4] Embden and Schliep, Centralbl. f. d. ges. Phys. u. Path. d. Stoffwechsel, 1907; Folin, Journ. of biol. Chem., **3**. In regard to the quantitative estimation of acetone see also O. Sammet, Zeitschr. f. physiol. Chem., **83**.

[5] Abderhalden's Handbuch der biochemischen Arbeitsmethoden, Bd. **3**.

for solutions of 1–11 per cent and has a disturbing action upon the determination of sugar by means of the polariscope. It is not precipitated by basic lead acetate or by ammoniacal lead acetate, neither does it ferment. On boiling with water, especially in the presence of a mineral acid, this acid decomposes into *α-crotonic acid*, which melts at 71–72° C., and water, $CH_3.CH(OH).CH_2.COOH = H_2O + CH_3.CH:CH.COOH$. It yields acetone on oxidation with a chromic-acid mixture.

Detection of β-Oxybutyric Acid in the Urine. If a urine is still levogyrate after fermentation with yeast, the presence of oxybutyric acid is probable. A further test may be made, according to KÜLZ, by evaporating the fermented urine to a syrup and, after the addition of an equal volume of concentrated sulphuric acid, distilling directly without cooling, α-crotonic acid is produced, which distills over, and, after collecting in a test-tube, crystals which melt at +72° C. separate on cooling. If no crystals are obtained, shake the distillate repeatedly with ether and let this spontaneously evaporate. The crystals which separate out can be purified according to EMBDEN and SCHMITZ by redissolving in ether, evaporating the chief part of the ether and precipitating with petroleum-ether, which removes the volatile fatty acids and benzoic acid.

The quantitative estimation is done by complete extraction of the β-oxybutyric acid by ether and determining the specific rotation. The extraction can be done according to MAGNUS-LEVY [1] or according to BERGELL.[2] Other methods of estimating β-oxybutyric acid have been suggested by DARMSTÄDTER, BOEKELMAN and BOUMA.[3] In regard to the quantitative estimation we refer to EMBDEN and SCHMITZ.[4]

EHRLICH'S [5] *Urine Test.* Mix 250 cc. of a solution which contains 50 cc. of HCl and 1 gram of sulphanilic acid in one liter, with 5 cc. of a ½ per cent solution of sodium nitrite (which produces very little of the active body, sulphodiazobenzene). In performing this test treat the urine with an equal volume of this mixture and then supersaturate with ammonia. Normal urine will become yellow or orange after the addition of ammonia (aromatic oxyacids may after a certain time give red azo bodies which color the upper layer of the phosphate sediment). In pathological urines there sometimes occurs (and this is the characteristic diazo reaction) a primary yellow coloration, with very marked secondary red coloration on the addition of ammonia, and the froth is also tinged with red. The upper layer of the sediment becomes greenish. The body which gives this reaction is unknown, but it especially occurs in the urine of typhoid patients (EHRLICH). Opinions differ in regard to the significance of this reaction. If the urine is made alkaline with sodium carbonate instead of ammonia and treated

[1] See Hoppe-Seyler, Thierfelder's Handbuch, 8. Aufl., 619, and Geelmuyden, Hammarsten's Festschr., 1906.

[2] Zeitschr. f. physiol. Chem., **33**.

[3] Darmstädter, *ibid.*, **37**; Boekelman and Bouma, see Maly's Jahresber, **31**.

[4] See footnote 5, page 825.

[5] Ehrlich, Zeitschr. f. klin. Med., **5**. See also Clemens, Deutsch. Arch. f. klin. Med., **63** (literature). Kutscher and Engeland, footnote 1, page 758.

with a freshly prepared solution of diazobenzene sulphonic acid made alkaline with sodium carbonate, normal urine also gives an orange or Bordeaux-red coloration. The known normal urinary constituents which give the diazo reaction are the aromatic oxyacids, antoxyproteic acid and the imidazole derivative found by ENGELAND (see page 758).

EHRLICH'S reaction with *p*-dimethylaminobenzaldehyde has already been discussed in connection with urobilinogen.

ROSENBACH'S urine test, which consists in adding nitric acid drop by drop to the boiling-hot urine and obtaining a claret-red coloration and a bluish-red foam on shaking, depends upon the formation of indigo substances, especially indigo-red.[1]

Fat in the Urine. The elimination of a urine which in appearance and richness in fat resembles chyle is called *chyluria.* It habitually contains a proteid and often fibrin. Chyluria occurs mostly in the inhabitants of the tropics. *Lipuria,* or the elimination of fat with the urine, may appear in apparently healthy persons, sometimes with and sometimes without albuminuria, in pregnancy, and also in certain diseases, as in diabetes, poisoning with phosphorus, and fatty degeneration of the kidneys.

Fat is usually detected by the microscope. It may also be dissolved with ether, and may invariably be detected by evaporating the urine to dryness and extracting the residue with ether.

Cholesterin is also sometimes found in the urine in chyluria and in a few other cases.

Amino-acids. Leucine and tyrosine have been repeatedly found in urine by the older methods, especially in acute yellow atrophy of the liver, in acute phosphorus poisoning, and in severe cases of typhoid and smallpox. Since β-naphthalene sulphochloride has been used in the detection of amino-acids these bodies have not only been repeatedly found in normal urine (glycocoll, see page 756), but also in pathological urines.[2]

Cystine. BAUMANN and GOLDMANN claim that a substance similar to cystine occurs in very small amounts in normal urine. This substance occurs in large quantities in the urine of dogs after poisoning with phosphorus. Cystine itself is only found with positiveness, and even then very rarely, in urinary calculi and in pathological urines, from which it may separate as a sediment. Cystinuria occurs oftener in men than in women. BAUMANN and V. UDRÁNSZKY found in urine in cystinuria the two diamines, *cadaverine* (pentamethylendiamine) and *putrescine* (tetramethylendiamine), which are produced in the putrefaction of proteins. Cases of cystinuria may occur with or without the occurrence of diamines in the urine, and only rarely are the diamines found in the urine as well as in the feces, which perhaps depends upon the fact, as found by CAMMIDGE and GARROD[3] in one case, that the diamines occur

[1] See Rosin, Virchow's Arch., **123**.

[2] Ignatowski, Zeitschr. f. physiol. Chem., **42**; Abderhalden and Schittenhelm, *ibid.*, **45**; Abderhalden and Barker, *ibid.*, **42**. See also footnote 5, page 756, and 2, 757.

[3] Baumann, Zeitschr. f. physiol. Chem., 8. In regard to the literature on cystinuria see Brenzinger, *ibid.*, **16**; Baumann and Goldmann, *ibid.*, **12**; Baumann and v. Udránszky, *ibid.*, **13**; Stadthagen and Brieger, Berlin. klin. Wochenschr., 1889; Cammidge and Garrod, Journ. of Path. and Bacteriol., 1900 (literature on diamines in the urine and feces); Loewy and Neuberg, Bioch. Zeitschr., **2**; Wolf and Schaffer, Journ. of biol. Chem., **4**; Williams and Wolf, *ibid.*, **6**.

only from time to time in the feces. Cystinuria is generally admitted as rather an anomaly in the protein metabolism where the cystine for unknown reasons is not destroyed as ordinarily. It is remarkable that the cystine of the food-proteins is eliminated by the urine, while in cystinurics, at least sometimes, such cystine introduced is quantitatively transformed.[1] Certain observations, such as the appearance of lysine in the urine of cystinurics (ACKERMANN and KUTSCHER [2]), make it probable that the demolition of other amino-acids is diminished in cystinuria. The properties and reactions of cystine have been given on pages 148 and 149.

Cystine is easily prepared from cystine calculi by dissolving them in alkali carbonate, precipitating the solution with acetic acid, and redissolving the precipitate in ammonia. The cystine crystallizes on the spontaneous evaporation of the ammonia. The cystine dissolved in the urine is detected, in the absence of proteid and sulphuretted hydrogen, by boiling with alkali and testing with a lead salt or sodium nitroprusside. To isolate cystine from the urine, acidify the urine strongly with acetic acid. The precipitate containing cystine is collected after twenty-four hours and digested with hydrochloric acid, which dissolves the cystine and calcium oxalate, leaving the uric acid undissolved. Filter, supersaturate the filtrate with ammonium carbonate, and treat the precipitate with ammonia, which dissolves the cystine and leaves the calcium oxalate. Filter again and precipitate with acetic acid. The precipitated cystine is identified by the microscope and the above-mentioned reactions. Cystine as a sediment is identified by the microscope. It must be purified by dissolving in ammonia and precipitating with acetic acid; it is then further tested. Traces of dissolved cystine may be detected by the production of benzoyl-cystine, according to BAUMANN and GOLDMANN. For the detection and estimation of cystine we can proceed to advantage in the following manner, suggested by GASKELL.[3] The urine freed from oxalates and phosphates by means of ammonia and calcium chloride is treated with an equal volume of acetone and with acetic acid. The crystals which precipitate are dissolved in ammonia and then purified by reprecipitation with acetone.

VII. URINARY SEDIMENTS AND CALCULI.

Urinary sediment is the more or less abundant deposit which is found in the urine after standing. This deposit may consist partly of organized and partly of non-organized constituents. The first, consisting of cells of various kinds, yeast-fungi, bacteria, spermatozoa, casts, etc., must be investigated by means of the microscope, and the following only applies to the non-organized deposits.

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[1] See Wolf and Schaffer, Journ. of biol. Chem., 4; and Hele, Journ. of Physiol., 39.

[2] Zeitschr. f. Biol., 57.

[3] Journ. of Physiol., 36.

As previously mentioned (page 674), the urine of healthy individuals may sometimes, even on voiding, be cloudy on account of the phosphates present, or become so after a little while because of the separation of urates. As a rule, urine just voided is clear, and after cooling shows only a faint cloud (nubecula) which consists of urine mucoid, a few epithelium-cells, mucous corpuscles, and urate particles. If an acid urine is allowed to stand, it will gradually change; it becomes darker and deposits a sediment consisting of uric acid or urates, and sometimes also calcium-oxalate crystals, in which yeast-fungi and bacteria are often to be seen. This change, which the earlier investigators called "ACID FERMENTATION OF THE URINE," is generally considered as an exchange of the dihydrogen alkali phosphates with the urates of the urine. Monohydrogen phosphates besides acid urates, quadriurates (page 708) or free uric acid or a mixture of both, according to conditions,[1] are thus formed.

Sooner or later, sometimes only after several weeks, the reaction of the original acid urine changes and becomes neutral or alkaline. The urine has now passed into the "ALKALINE FERMENTATION," which consists in the decomposition of the urea into carbon dioxide and ammonia by means of lower organisms, micrococcus ureæ, bacterium ureæ, and other bacteria. MUSCULUS[2] has isolated an enzyme from the micrococcus ureæ which decomposes urea, which is soluble in water and is called *urease*. During the alkaline fermentation volatile fatty acids, especially acetic acid, may be produced, chiefly by the fermentation of the carbohydrates of the urine (SALKOWSKI[3]). A fermentation by which nitric acid is reduced to nitrous acid, and another where sulphuretted hydrogen is produced, may sometimes occur.

When the alkaline fermentation has advanced only so far as to render the reaction neutral, there often occur in the sediment fragments of uric-acid crystals, sometimes covered with prismatic crystals of alkali urate; dark-colored spheres of ammonium urate, crystals of calcium oxalate, and sometimes crystallized calcium phosphate are also found. Crystals of ammonium-magnesium phosphate (triple phosphate) and spherical ammonium urate are specially characteristic of alkaline fermentation. The urine in alkaline fermentation becomes paler and is often covered with a fine membrane which contains amorphous calcium phosphate and glistening crystals of triple phosphate and numerous micro-organisms.

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[1] See Huppert-Neubauer, 10. Aufl., and A. Ritter, Zeitschr. f. Biologie, **35**.

[2] Musculus, Pflüger's Arch., **12**.

[3] Salkowski, Zeitschr. f. Physiol. Chem., **13**.

Non-Organized Sediments.

Uric Acid. This acid occurs in acid urines as colored crystals which are identified partly by their form and partly by their property of giving the murexid test. On warming the urine they are not dissolved. On the addition of caustic alkali to the sediment the crystals dissolve, and when a drop of this solution is placed on a microscope-slide and treated with a drop of hydrochloric acid, small crystals of uric acid are obtained which can be easily seen under the microscope.

Acid Urates. These occur only in the sediment of acid or neutral urines. They are amorphous, clay-yellow, brick-red, rose-colored, or brownish-red. They differ from other sediments in that they dissolve on warming the urine. They give the murexid test, and small microscopic crystals of uric acid separate on the addition of hydrochloric acid. Crystalline alkali urates occur very rarely in the urine, and as a rule only in such as have become neutral but not alkaline, by alkaline fermentation. The crystals are somewhat similar to those of neutral calcium phosphate; they are not dissolved by acetic acid, however, but give a cloudiness therewith due to small crystals of uric acid.

Ammonium urate may indeed occur as a sediment in a neutral urine which at first was strongly acid and has become neutralized by the alkaline fermentation, but it is only characteristic of ammoniacal urines. This sediment consists of yellow or brownish rounded spheres which are often covered with thorny-shaped prisms and, because of this, are rather large and resemble the thorn-apple. It reacts to the murexid test. It is dissolved by alkalies with the development of ammonia, and crystals of uric acid separate on the addition of hydrochloric acid to this solution.

Calcium oxalate occurs in the sediment generally as small, shining, strongly refractive quadratic octahedra, which on microscopical examination remind one of a letter-envelope. The crystals can only be mistaken for small, not fully developed crystals of ammonium-magnesium phosphate. They differ from these by their insolubility in acetic acid. The oxalate may also occur as flat, oval, or nearly circular disks with central cavities which from the side appear like an hour-glass. Calcium oxalate may occur as a sediment in an acid as well as in a neutral or alkaline urine. The quantity of calcium oxalate separated from the urine as sediment depends not only upon the amount of this salt present, but also upon the acidity of the urine. The solvent for the oxalate in the urine seems to be the diacid alkali phosphate, and the greater the quantity of this salt in the urine the greater the quantity of oxalate in solution. When, as previously mentioned (page 829), the simple-acid phosphate is formed from the diacid phosphate, on allowing the urine to stand, a corresponding part of the oxalate may be separated as sediment

Calcium carbonate occurs in considerable quantities as sediment in the urine of herbivora. It occurs in but small quantities as a sediment in human urine, and in fact only in alkaline urines. It either has the same appearance as amorphous calcium oxalate or it occurs as somewhat larger spheres with concentric bands. It dissolves in acetic acid with the generation of gas, which differentiates it from calcium oxalate. It is not yellow or brown like ammonium urate, and does not give the murexid test.

Calcium sulphate occurs very rarely as a sediment in strongly acid urine. It appears as long, thin, colorless needles, or generally as plates grouped together.

Calcium Phosphate. The CALCIUM TRIPHOSPHATE, $Ca_3(PO_4)_2$, which occurs only in alkaline urines, is always amorphous and occurs partly as a colorless, very fine powder, and partly as a membrane consisting of very fine granules. It differs from the amorphous urates in that it is colorless, dissolves in acetic acid, but remains undissolved on warming the urine. CALCIUM DIPHOSPHATE, $CaHPO_4+2H_2O$, occurs in neutral or only in very faintly acid urine.[1] It is found sometimes as a thin film covering the urine and sometimes as a sediment. In crystallizing, the crystals may be single, or they may cross one another, or they may be arranged in groups of colorless, wedge-shaped crystals whose wide end is sharply defined. These crystals differ from crystalline alkali urates in that they dissolve without a residue in dilute acids and do not give the murexid test.

Ammonium-magnesium phosphate, TRIPLE PHOSPHATE, may separate from an amphoteric urine in the presence of a sufficient quantity of ammonium salts, but it is generally characteristic of a urine which is ammoniacal through alkaline fermentation. The crystals are so large that they may be seen with the unaided eye as colorless glistening particles in the sediment, on the walls of the vessel, and in the film on the surface of the urine. This salt forms large prismatic crystals of the rhombic system (coffin-shaped) which are easily soluble in acetic acid. Amorphous *magnesium triphosphate,* $Mg_3(PO_4)_2$, occurs with calcium triphosphate in urines rendered alkaline by a fixed alkali. Crystalline magnesium phosphate, $Mg_3(PO_4)_2+22H_2O$, has been observed in a few cases in human urine (also in horse's urine) as strongly refractive, long rhombic plates.

As more rare sediments we find *cystine, tyrosine, hippuric acid, xanthine, hæmatoidine.* In alkaline urine blue crystals of *indigo* may also occur, due to a decomposition of indoxyl-glucuronic acid.

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[1] In regard to the conditions for the appearance of these sediments in urines see C. Th. Mörner, Zeitschr. f. physiol. Chem., 58.

Urinary Calculi.

Besides certain pathological constituents of the urine, all those urinary constituents which occur as sediments take part in the formation of urinary calculi. EBSTEIN[1] considers the essential difference between an amorphous and crystalline sediment in the urine on the one side and urinary sand or large calculi on the other to be the occurrence of an organic frame in the latter. As the sediments which appear in normal acid urine and in a urine alkaline through fermentation are diverse, so also are the urinary calculi which appear under corresponding conditions.

If the formation of the calculus and its further development take place in an undecomposed urine, it is called a PRIMARY formation. If, on the contrary, the urine has undergone alkaline fermentation and the ammonia formed thereby has given rise to a calculus formation by precipitating ammonium urate, triple phosphate, and earthy phosphates, then it is called a SECONDARY formation. Such a formation takes place, for instance, when a foreign body in the bladder produces catarrh accompanied by alkaline fermentation.

We discriminate between the nucleus or nuclei—if such can be seen—and the different layers of the calculus. The nucleus may be essentially different in different cases, for quite frequently it consists of a foreign body introduced in the bladder. The calculus may have more than one nucleus. In a tabulation made by ULTZMANN of 545 cases of vesicular calculi, the nucleus in 80.9 per cent of the cases consisted of uric acid (and urates); in 5.6 per cent, of calcium oxalate; in 8.6 per cent, of earthy phosphates; in 1.4 per cent, of cystein; and in 3.5 per cent, of some foreign body.

During the growth of a calculus it often happens that, for some reason or other, the original calculus-forming substance is covered with another layer of a different substance. A new layer of the original substance may deposit on the outside of this, and this process may be repeated. In this way a calculus consisting originally of a simple stone may be converted into a so-called compound stone with several layers of different substances. Such calculi are always formed when a primary is changed into a secondary formation. By the continued action of an alkaline urine containing pus, the primary constituents of a primary calculus may be partly dissolved and be replaced by phosphates. Metamorphosed urinary calculi are formed in this way.

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[1] Die Natur und Behandlung der Harnsteine. Wiesbaden, 1884.

Uric-acid calculi are very abundant. They are variable in size and form. The size of the bladder-stone varies from that of a pea or bean to that of a goose-egg. Uric acid stones are always colored; generally they are grayish-yellow, yellowish-brown, or pale red-brown. The upper surface is sometimes entirely even or smooth, sometimes rough or uneven. Next to the oxalate calculus the uric-acid calculus is the hardest. The fractured surface shows regular concentric, unequally colored layers which may often be removed as shells. These calculi are formed primarily. Layers of uric acid sometimes alternate with other layers of primary formation, most frequently with layers of calcium oxalate. The simple uric-acid calculus leaves very little residue when burnt on a platinum foil. It gives the murexid test, but there is no material development of ammonia when acted on by caustic soda.

Ammonium urate calculi occur as primary calculi in new-born or nursing infants, rarely in grown persons. They often occur as a secondary formation. The primary stones are small, with a pale yellow or dark-yellowish surface. When moist they are almost like dough; in the dry state they are earthy, easily crumbling into pale powder. They give the murexid test and develop much ammonia with caustic soda.

Calcium-oxalate calculi are, next to uric-acid calculi, the most abundant. They are either smooth and small (HEMP-SEED CALCULI) or larger, of the size of a hen's egg, with rough, uneven surface, or their surface is covered with prongs (MULBERRY CALCULI). These calculi produce bleeding easily, and therefore they often have a dark-brown surface due to decomposed blood-coloring matters. Among the calculi occurring in man these are the hardest. They dissolve in hydrochloric acid without developing gas, but are not soluble in acetic acid. After gently heating the powder, it dissolves in acetic acid with frothing. With more intense heat it becomes alkaline, due to the production of quicklime.

Phosphate Calculi. These, which consist mainly of a mixture of the normal phosphate of the alkaline earths with triple phosphate, may be very large. They are as a rule of secondary formation and contain besides these phosphates also some ammonium urate and calcium oxalate. These calculi ordinarily consist of a mixture of three constituents—earthy phosphate, triple phosphate, and ammonium urate—surrounding a foreign body as a nucleus. Their color is variable—white, dingy white, pale yellow, sometimes violet or lilac-colored (from indigo red). The surface is always rough. Calculi consisting of triple phosphate alone are seldom found. They are ordinarily small, with granular or radiated crystalline fracture. Stones of mono-acid calcium phosphate are also seldom obtained. They are white and have beautiful crystalline texture. The phosphatic calculi do not burn up, the powder dissolves in acid without effervescence, and the solution gives the reactions for phos-

phoric acid and the alkaline earths. The triple-phosphate calculi generate ammonia on the addition of an alkali.

Calcium-carbonate calculi occur chiefly in herbivora. They are seldom found in man. They have mostly chalky properties, and are ordinarily white. They are completely or in great part dissolved by acids with effervescence.

Cystine calculi occur but seldom. They are of primary formation, of various sizes, sometimes as large as a hen's egg. They have a smooth or rough surface, are white or pale yellow, and have a crystalline fracture. They are not very hard and are consumed almost entirely on the platinum foil, burning with a bluish flame. They give the above-mentioned reactions for cystine.

Xanthine calculi are very rarely found. They are also of primary formation. They vary from the size of a pea to that of a hen's egg. They are whitish, yellowish-brown or cinnamon-brown in color, of medium hardness, with amorphous fracture, and on rubbing appear like wax. They burn up completely when heated on a platinum foil. They give the xanthine reaction with nitric acid and alkali, but this must not be mistaken for the murexid test.

Urostealith calculi have been observed only a few times. In the moist state they are soft and elastic at the temperature of the body, but in the dry state they are brittle, with an amorphous fracture and waxy appearance. They burn with a luminous flame when heated on platinum foil and generate an odor similar to resin or shellac. Such a calculus, investigated by KRUKENBERG,[1] consisted of paraffin derived from a paraffin bougie used as a sound on the patient. Perhaps the urostealith calculi observed in other cases had a similar origin, although the substances of which they consisted have not been closely studied. HORBACZEWSKI has recently analyzed a case of urostealith which, to all appearances, was formed in the bladder. This calculus contained 25 p. m. water, 8 p. m. inorganic bodies, 117 p. m. bodies insoluble in ether, and 850 p. m. organic bodies soluble in ether, among which were 515 p. m. free fatty acids, 335 p. m. fat, and traces of cholesterin. The fatty acids consisted of a mixture of stearic, palmitic, and probably myristic acids.

HORBACZEWSKI[2] has also analyzed a bladder stone which contained 958.7 p. m. *cholesterin*.

Fibrin calculi sometimes occur. They consist of more or less changed fibrin-coagulum. On burning they develop an odor of burnt horn.

The *chemical investigation of urinary calculi* is of great practical importance. To make such an examination actually instructive it is necessary to investigate, separately, the different layers which constitute the calculus. For this purpose saw the calculus, previously wrapped in paper, with a fine saw so that the nucleus becomes accessible. Then peel off the different layers, or, if the stone is to be kept, scrape off enough of the powder from each layer for examination. This powder is then tested by heating on the platinum foil. It must not be forgotten that a calculus is never entirely burnt up, and also that it is never so free from organic matter that on heating it does not carbonize. Do not, therefore, lay too great stress on a very insignificant unburnt residue or on a very small

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[1] Chem. Untersuch. z. wissensch. Med., **2**. Cited from Maly's Jahresber., **19**, 422.

[2] Zeitschr. f. physiol. Chem., 18.

amount of organic matter, but consider the calculus in the former case as completely burnt and in the latter as unaffected.

When the powder is in great part burnt up, but a significant quantity of unburnt residue remains, then the powder in question contains as a rule urates mixed with inorganic bodies. In such cases remove the urate with boiling water and then test the filtrate for uric acid and the suspected bases. The residue is then tested according to the following *scheme* of HELLER, which is well adapted to the investigation of urinary calculi. In regard to the more detailed examination the reader is referred to special works on the subject.

On heating the powder on platinum foil, it

Does burn				Does not burn			
Without flame		With flame		The powder when treated with HCl			
The powder gives the murexid test	The powder when treated with KHO gives			Does not effervesce		Effervesces	
				The powder gently heated and treated with HCl			
				Effervesces		The powder when moistened with a little KHO	
						Effervesces	
	No noticeable ammonia reaction	Flame yellow, pale, continuous. Odor of resin or shellac on burning. Powder soluble in alcohol and ether	Flame yellow, continuous. Odor of burnt feathers. Insoluble in alcohol and ether. Soluble in KHO with heat. Precipitated herefrom by acetic acid and generation of H_2S	Effervesces	Uric acid	No NH_3, or at least only traces of NH_3. Powder dissolves in acetic acid or HCl. This solution is precipitated by ammonia (amorphous)	Abundant ammonia. The powder dissolves in acetic acid or HCl. This solution gives a crystalline precipitate with ammonia
Does not give the murexid test. The dried yellow residue becomes orange with alkali, beautiful red on warming	Strong ammonia reaction	Flame pale blue, burns a short time. Peculiar sharp odor. The powder dissolves in ammonia, and six-sided plates separate on the spontaneous evaporation of the ammonia			Ammonium urate		
					Xanthine		
					Cystine		
					Urostealith		
					Fibrin		
					Calcium carbonate		
					Calcium oxalate		
					Bone-earth (phosphate of calcium and magnesium)		
					Triple phosphate (mixed with unknown amount of earthy phosphate)		

CHAPTER XV

THE SKIN AND ITS SECRETIONS

In the structure of the skin of man and vertebrates many different kinds of substances occur which have already been considered, such as the constituents of the epidermal formation, the connective and fatty tissues, the nerves, muscles, etc. Among these the different horn structures, the hair, nails, etc., whose chief constituent, keratin, has been spoken of in another chapter (Chapter II), are of special interest.

The cells of the horny structure show, in proportion to their age, a different resistance to chemical reagents, especially fixed alkalies. The younger the horn-cell the less resistance it has to the action of alkalies; with advancing age the resistance becomes greater, and the cell-membranes of many horn-formations are nearly insoluble in caustic alkalies. Keratin (or the keratins) occurs in the horn structure mixed with other bodies, from which it is isolated with difficulty. These are detected by microchemical investigations, and according to Unna [1] three different substances can be detected in the horn substance, designated by him *A*-, *B*- and *C*-keratin.

The *A*-keratin, which forms the envelope of the horn and hair cells and the outer layer of the hair, is the purest keratin. It is not dissolved by fuming nitric acid at the ordinary temperature and does not give the xanthoproteic reaction, and its keratin nature is doubtful. The *B*-keratin, which occurs as the contents of the nail cells, gives the xanthoproteic reaction like the *C*-keratin occurring in hair, but differs from the *C*-keratin by being soluble in fuming nitric acid.

Besides these substances, which have been called keratins, the horn structure also contains other proteins which are soluble in pepsin-hydrochloric acid. Among these we find residue of nuclei and the so-called trichohyalin in the hair, which is a substance of unknown constitution and characterized by great insolubility. From these statements it is evident that we are here dealing with a mixture of different substances and for this reason it is unnecessary to give the older elementary analyses of the various epidermoidal structures.

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[1] Monatsch. f. prakt. Dermat., 44.

The quantity of sulphur and of mineral bodies is of certain interest. The sulphur and cystine content of these structures can be found on pages 113, 114 and in this connection it must be mentioned that, according to the investigations of RUTHERFORD and HAWK,[1] the sulphur content of human hair is higher in men than in women, at least for the Caucasian race, and also that red hair has the highest sulphur content irrespective of race or gender. Hair on incineration leaves considerable ash, which in human hair varies between 2.6 and 16 p. m., and in animal hair is still greater, even up to 71 p. m. in the hair of the deer. The ash consists of large amounts of alkali and calcium sulphate, and its sulphur probably originates from the organic substance, which make the statements as to the composition of the ash of hair of little value. Calcium occurs in larger amounts, especially phosphate as well as carbonate, and is most abundant in white hair. The amount of iron oxide in 1000 grams of the ash of human hair varies between 42.2 grams in blond and 108.7 grams in brown hair, and silicic acid between 66.1 grams in black and 424.6 grams in red hair (BAUDRIMONT). The nails are rich in calcium phosphate, and the feathers rich in silicic acid, especially the feathers of grain-eating birds. According to v. GORUP-BESANEZ[2] the quantity of silicic acid in grain-eating birds was 400 p. m., and in meat, berries and insect-eating birds the amount was only 270 p. m. of the total ash. DRECHSEL claims that at least a part of the silicic acid exists in the feathers in organic combination as an ester while according to CERNY[3] it exists only as an accidental contamination.

According to GAUTIER and BERTRAND[4] arsenic also occurs in the epidermal formations. GAUTIER says that arsenic is of importance in the formation and growth of the formations, and on the other hand the hair, nails, and epidermis-cells are of great importance in the excretion of arsenic.

The ability of the skin to take up chlorides as observed by WAHLGREN and by PADTBERG,[5] is remarkable. According to them the skin is an important chloride depot, which stores up chlorides when supplied in excess and gives them up when necessary.

The skin of invertebrates has been the subject, in a few cases, of chemical investigation, and in these animals various substances have been found, of which a few, though little studied, are worth discussing. Among them *tunicin*, which is found especially in the mantle of the

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[1] Journ. of Biol. Chem., **8**.
[2] Lehr. d. physiol. Chem., 4. Aufl., 660, 661; Baudrimont, *ibid.*
[3] Drechsel, Centralbl. f. Physiol., 11, 361; Cerny, Zeitschr. f. physiol. Chem., **62**.
[4] Gautier, Compt. Rend., **129**, **130**, **131**; Bertrand, *ibid.*, **134**.
[5] Wahlgren, Arch. f. exp. Path. u. Pharm., **61**; Padtberg, *ibid.*, **62**.

tunicata, and the widely diffused *chitin*, found in the cuticle-formation of invertebrates, are of interest.

Tunicin. Cellulose seems, from the investigations of AMBRONN, to occur rather extensively in the animal kingdom in the arthropoda and the mollusks. It has been known for a long time as the mantle of the *tunicata*, and this animal cellulose was called tunicin by BERTHELOT. According to the investigations of WINTERSTEIN there does not seem to exist any marked difference between tunicin and ordinary vegetable cellulose. On boiling with dilute acid, tunicin yields glucose, as shown first by FRANCHIMONT and later confirmed by WINTERSTEIN. By the action of acetic acid anhydride and sulphuric acid, upon tunicate-cellulose, ABDERHALDEN and ZEMPLÉN [1] obtained octoacetyl-cellobiose, which also indicates the relationship with the plant cellulose.

Chitin is not found in vertebrates. In invertebrates chitin is alleged to occur in several classes of animals; it occurs chiefly in cephalopods (sepia scales) and especially in the arthropods, in which it forms the chief organic constituent of the shells, etc. It has been found in the plant kingdom as in fungi (GILSON, WINTERSTEIN [2]). The question whether there are two or more chitins or whether there is only one is still disputed (KRAWKOW, ZANDER, WESTER [3]). No formula can be given for the same reasons (SUNDWIK, ARAKI, BRACH [4]).

Chitin is decomposed on boiling with mineral acids and yields, as shown by LEDDERHOSE, *glucosamine* and *acetic acid*. HOPPE-SEYLER and ARAKI found, on heating with alkali and a little water to 180°, that chitin was split into a new substance, *chitosan*, and acetic acid, and that this chitosan contained acetyl groups as well as glucosamine. FRÄNKEL and KELLY as well as OFFER [5] have obtained acetylglucosamine, $(C_6H_{12}NO_5)COCH_3$ and acetyldiglucosamine $(C_{12}H_{23}N_2O_9)COCH_3$ as cleavage products of chitin, and they consider chitin as a polymeric monacetyldiglucosamine.

The chitosan which v. FÜRTH and RUSSO [6] have obtained as a crystalline hydrochloric acid combination and which E. LOEWY has obtained as a crystalline sulphate is, according to the latter, a polymeric monacetyldiglucosamine with at least two monacetyldiglucosamine groups. Accord-

[1] Ambronn, Maly's Jahresber., **20**; Berthelot, Annal. de Chim. et Phys., **56**, Compt. Rend., **47**; Winterstein, Zeitschr. f. physiol. Chem., **18**; Franchimont, Ber. d. deutsch. chem. Gesellsch., **12**; Abderhalden and Zemplén, Zeitschr. f. physiol. Chem., **72**.

[2] Gilson, Compt. Rend., **120**; Winterstein, Ber. d. d. chem. Gesellsch., **27** and **28**.

[3] Krawkow, Zeitschr. f. Biol., **29**; Zander, Pflüger's Arch., **66**; Wester, Chem. Centralbl., 1909, II.

[4] Sundwik, Zeitschr. f. physiol. Chem., **5**; Araki, *ibid.*, **20**; Brach, Bioch. Zeitschr., **38**.

[5] Ledderhose, Zeitschr. f. physiol. Chem., **2** and **4**; Araki, l. c., Fränkel and Kelly, Monatshefte f. Chem., **23**; Offer, Bioch. Zeitschr., **7**.

[6] v. Fürth and Russo, Hofmeister's Beiträge, 8; Loewy, Bioch. Zeitschr., **23**; Brach, l. c.

ing to v. FÜRTH and RUSSO on cleavage it yields 25 per cent acetic acid and 60 per cent glucosamine. The formula is $(C_{28}H_{50}N_4O_{19})x$ according to v. FÜRTH and collaborators and splits according to the equation: $(C_{28}H_{50}N_4O_{19})_4+5xH_2O = 4x(C_6H_{13}NO_5)+2x(CH_3COOH)$. According to BRACH, who admits of at least four glucosamine groups in chitosan, the formula for chitin is $(C_{32}H_{54}N_4O_{21})x$ and contains 4 acetyl for every 4 nitrogen atoms. The transformation into chitosan consists in a rupture of one-half of the acetic acid groups in the chitin.

In a dry state chitin forms a white, brittle mass retaining the form of the original tissue. It is insoluble in boiling water, alcohol, ether, acetic acid, dilute mineral acids, and dilute alkalies. It is soluble in concentrated acids. It is dissolved without decomposing in cold concentrated hydrochloric acid, but is decomposed by boiling hydrochloric acid. According to KRAWKOW the various chitins behave differently with iodine or with sulphuric acid and iodine, in that some are colored reddish brown, blue, or violet, while others are not colored at all. According to WESTER chitin free from chitosan is not colored by iodine.

Chitin may be easily prepared from the wings of insects or from the shells of the lobster or the crab, the last-mentioned having first been extracted by an acid so as to remove the lime salts. The wings or shells are boiled with caustic alkali until they are white, afterward washed with water, then with dilute acid and water. The pigments remaining can be destroyed by permanganate. The excess of this last can be removed by a dilute solution of bisulphite, washed with water and then extracted with alcohol and ether.

Hyalin is the chief organic constituent of the walls of hydatid cysts. From a chemical point of view it stands close to chitin, or between it and protein. In old and more transparent sacs it is tolerably free from mineral bodies, but in younger sacs it contains a great quantity (16 per cent) of lime salts (carbonate, phosphate, and sulphate).

According to LÜCKE [1] its composition is:

	C	H	N	O
From old cysts	45.3	6.5	5.2	43.0
From young cysts	44.1	6.7	4.5	44.7

It differs from keratin on the one hand and from proteins on the other by the absence of sulphur, also by its yielding, when boiled with dilute sulphuric acid, a variety of sugar in large quantities (50 per cent), which is reducing, fermentable, and dextrogyrate. It differs from chitin by the property of being gradually dissolved by caustic potash or soda, or by dilute acids; also by its solubility on heating with water to 150° C.

The coloring matters of the skin and horn-formations are of different kinds, but have not been extensively studied. Those occurring in the stratum Malpighii of the skin, especially of the negro, and the black or brown pigment occurring in the hair, belong to the group of those substances which have received the name *melanins*.

[1] Virchow's Arch., 19.

Melanins. This group includes several different varieties of amorphous black or brown pigments which are insoluble in water, alcohol, ether, chloroform, and dilute acids, and which occur in the skin, hair, chorioidia, in sepia, in certain pathological formations, and in the blood and urine in disease. From the true native melanins we must differentiate the humus-like products obtained on boiling proteins with mineral acids and which have been called *melanoidins* or *melanoidic acid* (Schmiedeberg) and whose relation to the true melanins is still unknown.

The melanoidins are readily soluble in dilute alkali while the melanins show a different behavior in this regard. Of the melanins a few such as SCHMIEDEBERG'S *sarcomelanin*, and that from the melanotic sarcomata of horses, the *hippomelanin* (NENCKI, SIEBER, and BERDEZ), which are soluble with difficulty in alkalis, while others, such as the coloring matter of certain pathological swellings in man, the *phymatorhusin* (NENCKI and BERDEZ) are readily soluble in alkalies. The melanins, as above stated, are in general insoluble in dilute mineral acids; from black sheep-wool GORTNER [1] has isolated a melanin which was soluble in acetic acid and in dilute mineral acids (see below).

Among the melanins there are a few, for example the choroid pigment, which are free from sulphur (LANDOLT and others); others, on the contrary, as sarcomelanin and the pigment of the hair (SIEBER) are rather rich in sulphur (2–4 per cent), while the phymatorhusin found in certain swellings and in the urine (NENCKI and BERDEZ, K. MÖRNER) is very rich in sulphur (8–10 per cent). Whether any of these pigments, especially the phymatorhusin, contains any iron or not is an important though disputed point, for it leads to the question whether these pigments are formed from the blood-coloring matters.

According to NENCKI and BERDEZ the pigment, phymatorhusin, isolated by them from a melanotic sarcoma did not contain any iron, and according to them is not a derivative of hæmoglobin. K. MÖRNER and later also BRANDL and L. PFEIFFER found, on the contrary, that this pigment did contain iron, and they consider it as a derivative of the blood-pigments. The *sarcomelanin* (from a sarcomatous liver) analyzed by SCHMIEDEBERG contained 2.7 per cent iron which was partly in organic combination and could not be completely removed by dilute hydrochloric acid. The *sarcomelanic acid* prepared by SCHMIEDEBERG by the action of alkali on this melanin contained 1.07 per cent iron. The sarcomelanin investigated by ZDAREK and v. ZEYNEK also contained 0.4 per cent iron. Recently WOLFF [2] prepared two pigments from a melanotic liver, of which one was no doubt modified. The other, which was soluble in a soda solution, con-

[1] Gortner, Journ. of biol. Chem., 8, and Bioch. Bulletin, 1, 1911.

[2] Zdarek and v. Zeynek, Zeitschr. f. physiol. Chem., **36**; Wolff, Hofmeister's Beiträge, **5**. The literature on the melanins may be found in Schmiedeberg, "Elementarformeln einiger Eiweisskörper, etc." Arch. f. exp. Path. u. Pharm., **39**; also in Kobert, Wiener Klinik, **27** (1901), and Spiegler, Hofmeister's Beträge, **4**, and especially v. Fürth, Centralbl. f. allg. Path. u. Path. Anat., ,**15**, 1907, 617.

tained 2.51 per cent sulphur and 2.63 per cent iron, which was in great part split off by 20 per cent hydrochloric acid. From another liver he, on the contrary, obtained melanin free from iron but with 1.67 per cent sulphur. From this melanin he obtained, by treatment with bromine, a hydro-aromatic body which was related to xyliton (a condensation product of acetone). A similar product could not be obtained from the pigment of the hair (SPIEGLER) nor from hippomelanin (v. FÜRTH and JERUSALEM [1]).

The difficulties which attend the isolation and purification of the melanins have not been overcome in certain cases, while in others it is questionable whether the final product obtained has not another composition from the original coloring matter, owing to the energetic chemical processes resorted to in its purification. The elementary composition shows widely varying results in the different melanins, namely, 48–60 per cent carbon, and 8–14 per cent nitrogen. Under these circumstances, and as no doubt we have a large number of melanins having different composition, it seems that a tabulation of the analyses of the different preparations can only be of secondary importance.

GORTNER differentiates between two different groups of melanins. The one, to which the melanin isolated by him from sheeps-wool belongs, is soluble in very dilute acid, has a protein nature and is called *melanoprotein*. By the action of strong alkali the nitrogen and hydrogen content is much reduced and the quantity of carbon increased. The melanin is now insoluble in dilute acids, like the second group of melanins. The melanoprotein on hydrolysis with hydrochloric acid yields besides amino-acids, a black pigment, rich in carbon and insoluble in acids. The melanin isolated by PIETTRE [2] form sarcomatous horse tumors, on alkali hydrolysis, yielded amino-acids and a melanin much richer in carbon and poorer in nitrogen, a *melanin*. The sepia melanin and also the artificially prepared melanin by means of tyrosinase, had a similar behavior. The melanin is, therefore, according to PIETTRE, composed of a protein group and a pigment residue, which is insoluble in acids.

So little is known about the structural products of the melanins or melanoids that it is impossible to give the origin of these bodies. As undoubtedly there are several distinct melanins, their origin must also be distinct. The ferruginous melanins should be considered as originating from the blood-pigments until further research proves otherwise. Others, on the contrary, cannot have this origin; for example, the pigments of the hair and choroid, which are free from iron and which do not yield hæmopyrrol according to SPIEGLER. Several melanins—and this is also

[1] Wolff, Hofmeister's Beiträge, 5; Speigler, *ibid.*, 10; v. Fürth and Jerusalem, *ibid.*, 10.

[2] Gortner, l. c. and Bull. Soc. Chim. de France (4) 11; Piettre, Compt. Rend., 153. and Congrès, internat., de Path. Comparée, Paris, 1912.

true of the melanoids produced from proteins on cleavage with acids (SAMUELY [1])—yield indol or skatol and a pyrrol substance on fusion with alkali, while hippomelanin, according to v. FÜRTH and JERUSALEM, gives a fecal odor on this treatment, but does not yield any indol or skatol. More characteristic than the two last mentioned bodies is a phenol-like substance, which occurs to a slight extent, and gives a bluish-black color with ferric chloride (v. FÜRTH).

The cyclic complexes of the proteins are rightly considered as the mother-substance of the melanins (SAMUELY and v. FÜRTH and others), and this view has received support by the behavior of tyrosine with oxidases. It has been found that by the action of a plant oxidase, BERTRAND'S tyrosinase,[2] upon tyrosine, colored products and then melanin-like substances are formed. v. FÜRTH with SCHNEIDER and PRIBRAM, GESSARD, NEUBERG, DEWITZ and others [3] have shown that similar-acting tyrosinases also occur in the animal kingdom, in insects and sepia, in melanotic tumors and in pigmented skin, and v. FÜRTH and JERUSALEM have prepared an artificial melanin from tyrosine which shows great similarity to hippomelanin. Finally NEUBERG and JÄGER [4] have also prepared extracts from melanotic growths which formed a dark-brown pigment from adrenalin. As indicated above, we tend more and more to accept the view that the melanins are derived from the cyclic components of the proteins.

In addition to the coloring matters of the human skin it is in place here to treat of the pigments found in the skin or epidermal formation of animals.

The beautiful color of the feathers of many birds depends in certain cases on purely physical causes (interference-phenomena), but in other cases on coloring matters of various kinds. Such a coloring matter is the amorphous reddish-violet *turacin*, which contains 7 per cent copper and whose spectrum is very similar to that of oxyhæmoglobin. It must be remarked that according to LAIDLAW [5] turacin or at least a pigment with the same properties can be obtained on boiling hæmatoporphyrin in dilute ammonia with ammoniacal copper solution. KRUKENBERG [6] found a large number of coloring matters in bird's feathers, namely *zooerythrin*, *zoofulvin* *turacoverdin*, *zoorubin* *psittacofulvin*, and others which cannot be enumerated here.

Tetronerythrin, so named by WURM, is a red amorphous pigment which is soluble in alcohol and ether, and which occurs in the red warty spots over the eyes of the heathcock and the grouse, and which is very widely spread among the invertebrates (HALLIBURTON, DE MEREJKOWSKI MACMUNN). Besides tetronery-

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[1] Hofmeister's Beiträge, **2**.

[2] Compt. Rend., **122**.

[3] The literature can be found in v. Fürth and Jerusalem, Hofmeister's Beiträge, **10**.

[4] Neuberg, Virchow's Arch., **192**; Jäger, *ibid.*, **198**.

[5] Journ. of Physiol., **31**.

[6] Vergleichende physiol. Studien, Abth. **5**, and (2. Reihe) Abth. **1**, 151, Abth. **2**, 1, and Abth. **3**, 128.

thrin MacMunn found in the shells of crabs and lobsters a blue coloring matter, *cyanocrystallin*, which turns red with acids and by boiling water. *Hæmatoporphyrin*, according to MacMunn, also occurs in the integuments of certain of the lower animals. The blue pigment occurring in the fins of the fish, *crenilabrus pavo*, is according to v. Zeynek [1] a chromoprotein.

In certain butterflies (the pieridinæ) the white pigment of the wings consists, as shown by Hopkins,[2] of uric acid, and the yellow pigment of a uric-acid derivative, *lepidotic acid*, which yields a purple substance, *lepidoporphyrin*, on warming with dilute sulphuric acid. The yellow and red pigment of the Vanessa are, according to Linden,[3] of an entirely different kind. In this case we are dealing with a compound between protein and a pigment which is allied to bilirubin or urobilin, i.e., a compound similar to hæmoglobin.

In addition to the coloring matters thus far mentioned a few others found in certain animals (though not in the skin) will be spoken of.

Carminic Acid, or the red pigment of the cochineal, gives on oxidation, according to Liebermann and Voswinckel,[4] *cochenillic acid*, $C_{10}H_8O_7$, and *coccinic acid*. $C_8H_8O_6$, the first being the tri-carboxylic acid, and the other the di-carboxylic acid, of *m*-cresol. The beautiful purple solution of ammonium carminate has two absorption-bands between *D* and *E* which are similar to those of oxyhæmoglobin. These bands lie nearer to *E* and closer together and are less sharply defined. *Purple* is the evaporated residue from the purple-violet secretion, caused by the action of the sunlight, upon the so-called " purple gland " of the mantle of certain species of *murex* and *purpura*. According to Friedländer [5] the pigment is a bromine derivative of indigo and indeed di-bromindigo.

Among the remaining coloring matters found in invertebrates may be mentioned *blue stentorin*, *actiniochrom*, *bonellin*, *polyperythrin*, *pentacrinin*, *antedonin*, *crustaceorubin*, *janthinin*, and *chlorophyll*.

Sebum when freshly secreted is an oily semi-fluid mass which solidifies on the upper surface of the skin, forming a greasy coating. Röhmann and Linser hold that sebum is a mixture of the secretion of the sebaceous glands and of the constituents of the epidermis. Hoppe-Seyler found, in the sebum, a body similar to casein besides albumin and fat, while Röhmann and Linser claim that true fat occurs only to a very slight extent. On saponification the sebum gives an oil, *dermolein*, which combines readily with iodine, and another body, *dermocerin*, which melts at 64–65° and which occurs to a considerable extent in dermoid cysts, and which is perhaps identical with the constituent of cysts, called cetyl alcohol by v. Zeynek. According to Ameseder this dermocerin is not a pure substance, and the cetyl alcohol obtained from the fat of dermoid cysts is an *eicosyl alcohol*, $C_{20}H_{42}O$, corresponding to arachinic acid. Cholesterin is found in especially large quantities in

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[1] Wurm, cited from Maly's Jahresber., 1; Halliburton, Journ. of Physiol., 6; Merejkowski, Compt. Rend., 93; MacMunn, Proc. Roy. Soc., 1883, and Journ. of Physiol., 7; v. Zeynek, Zeitschr. f. physiol. Chem., 84 and 86, and Wien. Sits.-Ber. 121, 1912.

[2] Phil. Trans., 186.

[3] Pflüger's Arch., 98.

[4] Ber. d. deutsch. chem. Gesellsch., 30.

[5] *Ibid.*, 42.

the *vernix caseosa*. RÜPPEL[1] found on an average in the *vernix caseosa* 348.52 p. m. water and 138.72 p. m. ether extractives, and also mentions the presence of isocholesterin. These claims are disputed by UNNA.[2] In his experience isocholesterin does not occur in the vernix fat nor in the sebum of man, although all kinds of sebum contain cholesterin.

According to UNNA and GOLODETZ[3] the fat secretion (of the skin), as the fat of the ball of the foot, and sebum are rich in oxycholesterin, while the cell fats of the outer skin does not contain any oxycholesterin. The nails, which are rather rich in oxycholesterin, are an exception.

On account of the opinion generally held that the wax of the plant epidermis serves as protection for the inner parts of the fruit and plant, LIEBREICH[4] has suggested that these combinations of fatty acids with monatomic alcohols are the cause of the waxes having a greater resistance as compared with the glycerin fats. He also considers that the cholesterin fats play the rôle of a protective fat in the animal kingdom, and he has been able to detect cholesterin fat in human skin and hair, in *vernix caseosa*, whalebone, tortoise-shell, cow's horn, the feathers and beaks of several birds, the spines of the hedgehog and porcupine, the hoofs of horses, etc. He draws the following conclusion from this, namely, that the cholesterin fats always appear in combination with the keratinous substance, and that the cholesterin fat, like the wax of plants, serves as protection for the skin-surface of animals. Of the sebum fats investigated by UNNA all contained, with the exception of the epidermis fat, besides cholesterin, greater or smaller amounts of cholesterin ester. The epidermis fat, on the contrary, was almost free from esters and consisted chiefly of free cholesterin.

In the fatty protective substance secreted by the *Psylla alni*, SUNDVIK[5] found psylla-alcohol, $C_{33}H_{68}O$, which exists there as an ester in combination with psyllic acid, $C_{32}H_{65}COOH$. This alcohol has also been found in the wax of the humble-bee.

Cerumen is a mixture of the secretion of the sebaceous and sweat glands of the cartilaginous part of the outer passages of the ear. It chiefly contains soaps and fat, fatty acids, cholesterin and protein, and besides these a red substance easily soluble in alcohol and with a bitter-sweet taste.[6]

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[1] Hoppe-Seyler, Physiol. Chem., 760; Linser with Röhmann, Centralbl. f. Physiol., **19**, 317; see also reference in *ibid.*, 18, from Deutsch. Arch. f. klin. Med., 1904; Rüppel, Zeitschr. f. physiol. Chem., **21**; Ameseder, *ibid.*, **52**; Zumbusch, *ibid.*, **59**.

[2] Monatsch. f. prakt. Dermat., **45**.

[3] Bioch. Zeitschr., **20**.

[4] Virchow's Arch., **121**.

[5] Zeitschr. f. physiol. Chem., **17**, **25**, **32**, **53**, **54** and **72**.

[6] See Lamois and Martz, Maly's Jahresber., **27**, 40.

The **preputial secretion,** *smegma præputii*, contains chiefly fat, also cholesterin and ammonium soaps, which probably are produced from decomposed urine. The hippuric acid, benzoic acid, and calcium oxalate found in the smegma of the horse probably have the same origin.

We may also consider as a preputial secretion the *castoreum*, which is secreted by two peculiar glandular sacs, in the prepuce of the beaver. The castoreum is a mixture of proteins, fats, resins, traces of phenol (volatile oil), and a non-nitrogenous body, *castorin*, crystallizing from alcohol in four-sided needles, insoluble in cold water, but somewhat soluble in boiling water, and whose composition is little known.

In the secretion from the anal glands of the skunk, butyl mercaptan and alkyl sulphides have been found (ALDRICH, E. BECKMANN [1]).

Wool-fat, or the so-called fat-sweat of sheep, is a mixture of the secretion of the sudoriparous and sebaceous glands. There is found in the watery extract a large quantity of potassium which is combined with organic acid, volatile and non-volatile fatty acids, benzoic acid, phenol-sulphuric acid, lactic acid, malic acid, succinic acid, and others. The fat contains, among other bodies, abundant quantities of ethers of fatty acids with cholesterin and isocholesterin. DARMSTÄDTER and LIFSCHÜTZ have found other alcohols in wool-fat besides myristic acid, also two oxyfatty acids, *lanoceric acid*, $C_{26}H_{50}O_4$, and *lanopalmitic acid*, $C_{16}H_{32}O_3$. Isocholesterin, oxycholesterin and carnaubyl alcohol, $C_{24}H_{49}OH$, are besides the two last-mentioned acids, substances that are characteristic of wool-fat. According to RÖHMANN [2] wool-fat contains a body *lanocerin*, which is the internal anhydride of the above-mentioned lanoceric acid.

The secretion of the coccygeal glands of ducks and geese contains a body similar to casein, besides albumin, nuclein, lecithin, and fat, but no sugar (DE JONGE). The chief constituent is *octadecyl alcohol*, $C_{18}H_{38}O$, which represents 40–45 per cent of the ethereal extract (RÖHMANN). The fatty acids are oleic acid, small amounts of caprylic acid, palmitic acid, and stearic acid, and optical isomers of lauric and myristic acid. The fatty acids are in great part combined with the octadecylic alcohol, and this is probably formed by the reduction of stearic acid or oelic acid. The secretion also contains a substance related to lanocerin which RÖHMANN calls *pennacerin*. Poisonous bodies have been found in the secretion of the skin of the salamander and the toad, namely, *samandarin* (ZALESKI, FAUST) and *bufidin* (JORNARA and CASALI), *bufotalin* and the disputed bodies *bufonin* and *bufotenin* (FAUST, BERTRAND and PHISALIX [3]). The active constituents in the poison of the rattle-snake and cobra, the *crotalotoxin* and the *ophiotoxin* have been isolated and studied by FAUST.[4] They are free from nitrogen and have a similar composition, namely, $C_{34}H_{54}O_{21}$ and $C_{34}H_{52}O_{20}$ and are classified in the pharmacological group of *sapotoxins* by FAUST. *Thalassin* is the crystalline body discovered by RICHET [5] which is the poisonous constituent of the feelers of the sea nettle.

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[1] Aldrich, Journ. of Exp. Med., 1; Beckmann, Maly's Jahresber., 26, 566.

[2] Darmstäder and Lifschütz, Ber. d. d. Chem., Gesellsch., 29 and 31; Röhmann, Hofmeister's Beiträge, 5, and Centralbl. f. Physiol., 19, 317. See also Unna, l. c., 45; and Lifschütz and Unna, *ibid.*, p. 234.

[3] De Jonge, Zeitschr. f. physiol. Chem., 8; Röhmann, l. c.; Zaleski, Hoppe-Seyler's Med.-chem. Untersuch., p. 85; Faust, Arch. f. exp. Path. u. Pharm., 41; Jornara and Casali, Maly's Jahresbr., 8; Faust, Arch. f. exp. Path. u. Pharm., 47 and 49; Bertrand, Compt. Rend., 135; Bertrand and Phisalix, *ibid.*

[4] Arch. f. exp. Path. u. Pharm., 56 and 64.

[5] Pflüger's Arch., 108.

The Perspiration. Of the bodies secreted by the skin, whose quantity amounts to about $\frac{1}{64}$ of the weight of the body, a disproportionately large part consists of water. Next to the kidneys, the skin, in man, is the most important means for the elimination of water. As the glands of the skin and the kidneys stand near to each other in regard to their functions, they may to a certain extent act vicariously.

The circumstances which influence the secretion of perspiration are numerous, and the quantity of sweat secreted must consequently vary considerably. The secretion differs in different parts of the skin, and it has been stated that the perspiration of the cheek, that of the palm of the hand, and that under the arm stand to each other as 100:90:45. From the unequal secretion on different parts of the body it follows that no results as to the quantity of secretion for the entire surface of the body can be calculated from the quantity secreted by a small part of the skin in a given time. In determining the total quantity a stronger secretion is as a rule produced, and as the glands can with difficulty work for a long time with the same energy, it is hardly correct to estimate the quantity of secretion per day from a strong secretion during only a short time.

The perspiration obtained for investigation is never quite pure, but contains cast-off epidermis-cells, also cells and fat-globules from the sebaceous glands. Filtered perspiration is a clear, colorless fluid with a salty taste and of different odors from different parts of the body. The physiological reaction is acid, according to most reports. Under certain conditions an alkaline sweat may be secreted (Trümpy and Luchsinger, Heuss). An alkaline reaction may also depend on a decomposition with the formation of ammonia. According to a few investigators the physiological reaction is alkaline, and an acid reaction depends upon an admixture of fatty acids from the sebum. Camerer found that the reaction of human perspiration in certain cases was acid and in others alkaline. Moriggia found that the sweat from herbivora was ordinarily alkaline, while that from carnivora was generally acid. Smith [1] showed that horse's sweat is strongly alkaline.

Kittsteiner,[2] who has found that human perspiration is nearly always acid, has also found that the perspiration from the vola manus, when not contaminated with sebum, is acid in reaction and that an acid reaction is not necessarily dependent upon an admixture with sebum.

The specific gravity of human perspiration varies between 1.001 and 1.010. It contains 977.4–995.6 p. m., average about 982 p. m. water. The solids are 4.4–22.6 p. m. The molecular concentration also varies widely and the freezing-point depression depends essentially

[1] Trümpy and Luchsinger, Pflüger's Arch., 18; Heuss, Maly's Jahresber., 22; Camerer, Zeitschr. f. Biologie, 41; Moriggia, Moleschott's Untersuch. zur Naturlehre, 11; Smith, Journ. of Physiol., 11. In regard to the older literature on perspiration, see Hermann's Handbuch, 5, Thl. 1, 421 and 543.

[2] Arch. f. Hyg., 73 and 78.

upon the content of NaCl. ARDIN-DELTEIL found $\Delta = -0.08$–$0.46°$, average $-0.327°$. BRIEGER and DISSELHORST found with perspiration containing 2.9, 7.07 and 13.5 p. m. NaCl that the Δ was equal to $-0.322°$, $-0.608°$ and $-1.002°$, respectively. TARUGI and TOMASINELLI [1] found Δ to be 0.52° as an average. KITTSTEINER[2] found that perspiration had an average specific gravity of 1.0046 and the average quantities of nitrogen and sulphur were 0.5 and 0.08 p. m. respectively. The NaCl content increased with the rapidity of secretion while the nitrogen content diminished. The organic bodies are *neutral fats*, *cholesterin*, *volatile fatty acids*, traces of *protein* (according to LECLERC and SMITH always in horses, and according to GAUBE regularly in man, while LEUBE [3] claims only occasionally after hot baths, in BRIGHT'S disease, and after the use of pilocarpin), *creatinine* (CAPRANICA), *aromatic oxyacids*, *ethereal-sulphuric acids* of *phenol* and *skatoxyl* (KAST [4]), sometimes also of *indoxyl*, *serine* (page 145) and lastly *urea*. The quantity of urea has been determined by ARGUTINSKY. In two steam-bath experiments, in which in the course of $\frac{1}{2}$ and $\frac{3}{4}$ hour respectively he obtained 225 and 330 cc. of perspiration, he found 1.61 and 1.24 p. m. urea. Of the total nitrogen of the perspiration in these two experiments 68.5 per cent and 74.9 per cent respectively belong to the urea. From ARGUTINSKY'S experiments, and also from those of CRAMER,[5] it follows that of the total nitrogen a portion, not to be disregarded, is eliminated by the perspiration. This portion was indeed 12 per cent, in an experiment of CRAMER, at high temperature and powerful muscular activity, and ZUNTZ and his collaborators find indeed more than 13 per cent in high altitudes. CRAMER also found ammonia in the perspiration. In uræmia and in anuria in cholera. urea may be secreted in such quantities, by the sweat-glands, that crystals deposit upon the skin. The mineral bodies consist chiefly of sodium chloride with some potassium chloride, alkali sulphate and phosphate. The relative quantities of these in perspiration differ materially from the amount in the urine (FAVRE, KAST [6]). The relation, according to KAST, is as follows:

	Chlorine	Phosphate	Sulphate:
In perspiration.....	1	0.0015	0.009
In urine.....	1	0.1320	0.397

[1] Ardin-Delteil, Maly's Jahresber., **30**; Brieger and Disselhorst, Deutsch. med. Wochenschr., **29**; Tarugi and Tomasinelli, cited in Physiol. Centralbl., **22**, 748.

[2] l. c.

[3] Leclerc, Compt. Rend., **107**; Gaube, Maly's Jahresber., **22**; Leube, Virchow's Arch., **48** and **50**, and Arch. f. klin. Med., **7**.

[4] Capranica, Maly's Jahresber., **12**; Kast, Zeitschr. f. physiol. Chem., **11**.

[5] Argutinsky, Pflüger's Arch., **46**; Cramer, Arch. f. Hygiene, **10**.

[6] Compt. Rend., **35**, and Arch. génér. de Med. (5), **2**; Kast, l. c.

KAST found that the proportion of ethereal-sulphuric acid to the sulphate-sulphuric acid in perspiration was 1:12. After the administration of aromatic substances the ethereal-sulphuric acid does not increase to the same extent in the perspiration as in the urine (see Chapter XIV). The quantity of mineral substances was on an average 7 p. m.

Sugar may pass into the perspiration in diabetes, but the passage of the bile-coloring matters has not been positively shown in this secretion. *Benzoic acid, succinic acid, tartaric acid, iodine, arsenic, mercuric chloride* and *quinine* pass into the perspiration. *Uric acid* has also been found in the perspiration in gout and *cystine* in cystinuria.

Chromhidrosis is the name given to the secretion of colored perspiration. Sometimes perspiration has been observed to be colored blue by indigo (BIZIO), by pyocyanin, or by ferro-phosphate (COLLMANN [1]). True blood-sweat, in which blood-corpuscles exude from the opening of the glands, has also been observed.

The *exchange of gas through the skin* is of great importance for non-scaly amphibians; in mammalia, birds and human beings it is of little importance compared with the exchange of gas by the lungs. The absorption of oxygen by the skin, which was first shown by REGNAULT and REISET, is small, and according to ZUELZER amounts under the most favorable circumstances to $\frac{1}{100}$ of the oxygen absorbed by the lungs. The quantity of carbon dioxide eliminated by the skin increases with the rise of temperature (AUBERT, RÖHRIG, FUBINI and RONCHI, BARRATT and according to WILLEBRAND beginning at 33°)[2]. It especially increases with hyperæmia of the skin and in particular after muscular activity. It is also greater in light than in darkness. It is greater during digestion than when fasting, and greater after a vegetable than after an animal diet (FUBINI and RONCHI). The quantity calculated by different investigators for the entire skin surface in twenty-four hours varies between 2.23 and 32.8 grams. According to SCHIERBECK and WILLEBRAND [3] the average quantity is 7.5–9 grams, and it is ordinarily given as about 1.5 per cent of the quantity eliminated by the lungs. In a horse, ZUNTZ, with LEHMANN and HAGEMANN,[4] found for twenty-four hours an elimination of carbon dioxide by the skin and intestine which amounted to nearly 3 per cent of the total respiration. Less than four-fifths of this carbon dioxide came from the skin respiration. The same investigators found that the skin respiration equals $2\frac{1}{2}$ per cent of the simultaneous lung respiration.

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[1] Bizio, Wien. Sitzungsber., **39**; Collmann, cited from v. Gorup-Besanez's Lehrbuch, 4. Aufl., 555.

[2] Zuelzer, Zeitschr. f. klin. Med., **58**; Aubert, Pflüger's Arch., **6**; Röhrig, Deutsch. Klin., 1872, 209; Fubini and Ronchi, Moleschott's Untersuch. z. Naturlehre, **12**; Barratt, Journ. of Physiol., **21**; Willebrand, Skand. Arch. f. Physiol., **18**.

[3] See Hoppe-Seyler, Physiol. Chem., 580; Schierbeck, Arch. f. (Anat. u.) Physiol., 1892; Willebrand, l. c.

[4] Arch. f. (Anat. u.) Physiol., 1894, and Maly's Jahresber., **24**.

CHAPTER XVI.

RESPIRATION AND OXIDATION.

DURING life a constant exchange of gases takes place between the animal body and the surrounding medium. Oxygen is inspired and carbon dioxide expired. This exchange of gases, which is called respiration, is brought about in man and vertebrates by the nutritive fluids, blood and lymph, which circulate in the body and which are in constant communication with the outer medium on one side and the tissue-elements on the other. Such an exchange of gaseous constituents may take place wherever the anatomical conditions offer no obstacle, and in man it may go on in the intestinal tract, through the skin, and in the lungs. As compared with the exchange of gas in the lungs, the exchange already mentioned, which occurs in the intestine and through the skin, is very insignificant. For this reason we will discuss in this chapter only the exchange of gas between the blood and the air of the lungs on one side and the blood and lymph and the tissues on the other. The first is often designated as external respiration, and the other, internal respiration. Besides this we will discuss the oxidation processes caused by the internal respiration.

I. THE GASES OF THE BLOOD.

Since the pioneer investigations of MAGNUS and LOTHAR MEYER, the gases of the blood have formed the subject of repeated careful investigations by prominent experimenters, among whom must be mentioned first C. LUDWIG and his pupils, and E. PFLÜGER and his school; and C. BOHR. By these investigations not only has science been enriched by a mass of facts, but also the methods themselves have been made more perfect and accurate. In regard to these methods, as also in regard to the laws of the absorption of gases by liquids, dissociation, and related questions, the reader is referred to text-books on physiology, on physics, and on gasometric analysis.

The gases occurring in blood under physiological conditions are *oxygen*, *carbon dioxide* and *nitrogen*, and traces of argon, and perhaps also carbon monoxide. Traces of hydrogen and marsh-gas also sometimes occur. The nitrogen is found only in very small quantities, on an average 1.2 vols. per cent. The quantity is here, as in all following

experiments, calculated for 0° C. and 760 mm. mercury pressure. The nitrogen seems to be simply absorbed by the blood, at least in great part. It appears, like argon, to play no direct part in the processes of life, and its quantity varies but slightly in the blood of different blood-vessels.

The oxygen and carbon dioxide behave otherwise, as their quantities have significant variations, not only in the blood from different blood-vessels, but also because many factors, such as a difference in the rapidity of circulation and the ventilation of the lungs, a different temperature, alkalinity of the blood, rest and activity cause a change. In regard to the gases they contain, the greatest difference is observable between the blood of the arteries and that of the veins.

The *quantity of oxygen* in the arterial blood (of dogs) is on an average 22 vols. per cent (PFLÜGER, BOHR and HENRIQUES). In human blood SETSCHENOW found about the same quantity, namely, 21.6 vols. per cent. LEOWY in another manner has determined the quantity of oxygen which the blood can take up by first shaking human venous blood with air and then calculating from this the quantity of oxygen in human arterial blood. He calculates the average amount as 18 vols. per cent. Lower figures have been found for the blood of herbivora (such as horse, sheep, rabbits) and birds (hen and ducks) namely, 14–10.7 per cent (ZUNTZ and HAGEMANN, SCZELKOW, WALTER, JOLYET). Venous blood in different vascular regions has variable quantities of oxygen. By summarizing a great number of analyses by different experimenters, ZUNTZ has calculated that the venous blood of the right side of the heart contains on an average 7.15 per cent less oxygen than the arterial blood.

The *quantity of carbon dioxide* in the arterial blood (of dogs) is about 40 vols. per cent (LUDWIG, SETSCHENOW, PFLÜGER, P. BERT, BOHR and HENRIQUES and others), or a little above. In herbivora and the above-mentioned birds the quantity of carbon dioxide in the arterial blood is higher than in the carnivorous dog. SETSCHENOW found 40.3 vols. per cent in human arterial blood. The quantity of carbon dioxide in venous blood varies still more (LUDWIG, PFLÜGER, and their pupils, P. BERT, MATHIEU and URBAIN, and others). According to the calculations of ZUNTZ, the venous blood of the right side of the heart contains about 8.2 per cent more carbon dioxide than the arterial. The average amount may be put down as 50 vols. per cent. HOLMGREN found in blood after asphyxiation even 69.21 vols. per cent carbon dioxide.[1]

[1] All the figures given above may be found in Zuntz's "Die Gase des Blutes" in Hermann's Handbuch d. Physiol., 4, Thl. 2, 33–43, which also contains detailed statements and the pertinent literature, and Bohr in Nagel's Handbuch der Physiologie des Menschen, Bd. 1, Hefte 1, 1905, and in Loewy, Handb. d. Bioch. of C. Oppenheimer, Bd. 4.

Oxygen is dissolved only in a small extent by the plasma, whose absorbability for oxygen is 97.5 per cent of that of water, according to BOHR. The greater part or nearly all of the oxygen is loosely combined with the hæmoglobin. The quantity of the oxygen which is contained in the blood of the dog corresponds closely to the quantity which, from the activity of the hæmoglobin, we should expect to combine with oxygen and from the quantity of hæmoglobin contained therein. It is difficult to ascertain how far the circulating arterial blood is saturated with oxygen, as immediately after bleeding a loss of oxygen always takes place. Still it seems to be unquestionable that it is not quite completely saturated with oxygen, in life. The laws which regulate the binding of the oxygen in the blood will be found in the discussion of the gas exchanged between the blood and the air of the lungs.

The carbon dioxide of the blood occurs in part, and indeed, according to the investigations of ALEX. SCHMIDT,[1] ZUNTZ,[2] and L. FREDERICQ,[3] to the extent of at least one-third in the blood-corpuscles, also in part, and in fact the greatest part, in the plasma or serum. BOHR[4] claims that about 30 mm. may be considered as the average pressure of the carbon dioxide in the organism, and with such a pressure the quantity of physically dissolved CO_2 in 100 cc. of the blood amounts to 2.01 cc. As the blood with this tension takes up about 40 vols. per cent CO_2, therefore about 5 per cent of the total carbon dioxide is simply dissolved. Under the assumption that the blood corpuscles make up about one-third of the volume of the blood, of the physically dissolved CO_2, 0.59 cc. exists with the corpuscles and 1.42 cc. with the plasma.

As the blood corpuscles in 100 cc. blood as above stated take up at the above pressure about 14 cc. CO_2, only a small part of its CO_2 is physically dissolved. The chief mass of the CO_2 is loosely combined and the constituent of these cells which unites with the CO_2 seems to be the alkali combined with phosphoric acid, oxyhæmoglobin or hæmoglobin, and globulin on one side and the hæmoglobin itself on the other. That in the red blood-corpuscles alkali phosphate occurs in such quantities that it may be of importance in the combination with carbon dioxide is not to be doubted; and it must be allowed that from the diphosphate, by a greater partial pressure of the carbon dioxide, monophosphate and alkali carbonate are formed, while by a lower partial pressure of the carbon dioxide, the mass action of the phosphoric acid again comes into play, so that, with the carbon dioxide becoming free, a reformation of

[1] Ber. d. k. sächs. Gesellsch. d. Wissensch. math.-phys. Klasse, 1867.

[2] Centralbl. f. d. med. Wissensch., 1867, 529.

[3] Recherches sur la constitution du Plasma sanguin, 1878, 50, 51.

[4] In regard to the work of Bohr we will refer here and in future to Nagel's Handbuch der Physiologie des Menschen, Bd. 1.

alkali diphosphate takes place. It is generally admitted that the blood-coloring matters, especially the oxyhæmoglobin, which can expel carbon dioxide from sodium carbonate *in vacuo*, acts like an acid, and as the globulins also act similarly (see below), these bodies may also occur in the blood-corpuscles as an alkali combination. The alkali of the blood-corpuscles must, therefore, according to the law of mass action, be divided between the carbon dioxide, phosphoric acid, and the other constituents of the blood-corpuscles which possess acidic properties, and among these especially the blood pigments, because the globulin can hardly be of importance on account of its small quantity. By greater mass action or greater partial pressure of the carbon dioxide, bicarbonate must be formed at the expense of the diphosphates and the other alkali combinations, while at a diminished partial pressure of the same gas, with the escape of carbon dioxide, the alkali diphosphate and the other alkali combinations must be reformed at the cost of the bicarbonate.

Hæmoglobin must nevertheless, as the investigations of SETSCHENOW [1] and ZUNTZ, and especially those of BOHR and TORUP,[2] have shown, be able to hold the carbon dioxide loosely combined even in the absence of alkali. BOHR has also found that the dissociation curve of the carbon dioxide hæmoglobin corresponds essentially to the curve of the absorption of carbon dioxide, on which ground he and TORUP consider the hæmoglobin itself as of importance in the binding of the carbon dioxide of the blood, and not its alkali combinations. According to BOHR the hæmoglobin takes up the two gases, oxygen and carbon dioxide, simultaneously by the oxygen uniting with the pigment nucleus and the carbon dioxide with the protein component. But as according to the researches of ZUNTZ [3] the combination of hæmoglobin with the alkali is first split to any great extent with a carbon dioxide tension of more than 70 mm., it must be admitted that with the ordinary CO_2 pressure in the organism, the combination of the carbon dioxide in the blood corpuscles does not essentially take place through the agency of the alkali but chiefly by means of the hæmoglobin.

The chief part of the carbon dioxide of the blood is found in the blood-plasma or the blood-serum, which follows from the fact that the serum is richer in carbon dioxide than the corresponding blood itself. By experiments with the air-pump on blood-serum it has been found that the chief part of the carbon dioxide contained in the serum is given off in a vacuum, while a smaller part can be removed only after the

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[1] Centralbl. f. d. med. Wissench., 1877. See also Zuntz in Hermann's Handbuch, 76.

[2] Zuntz, l. c., 76; Bohr, Maly's Jahresber., 17; Torup, *ibid.*

[3] Centralbl. f. d. med. Wissensch., 1867.

addition of an acid. The red blood-corpuscles also act as an acid, and therefore in blood all the carbon dioxide is expelled *in vacuo*. Hence a part of the carbon dioxide is in firm chemical combination in the serum.

Absorption experiments with blood-serum have shown us further that the carbon dioxide which can be pumped out is in greater part loosely chemically combined, and from this loose combination of the carbon dioxide it necessarily follows that the serum must also contain simply absorbed carbon dioxide. For the form of binding of the carbon dioxide contained in the serum or the plasma, there are the three following possibilities: 1. A part of the carbon dioxide is simply absorbed; 2. Another part is in loose chemical combination; 3. A third part is in firm chemical combination.

The quantity of physically dissolved carbon dioxide in the serum cannot be higher than about 2 vols. per cent, as the quantity of carbon dioxide in the plasma corresponding to 100 cc. of blood is given above as 1.42 cc.

The quantity of carbon dioxide in the blood-serum which is combined as a firm chemical union depends upon the quantity of simple alkali carbonate in the serum. This amount is not known, and it cannot be determined either by the alkalinity found by titration, nor can it be calculated from the excess of alkali found in the ash, because the alkali is not only combined with carbon dioxide, but also with other bodies, especially with protein. The quantity of carbon dioxide in firm chemical combination cannot be ascertained after pumping out *in vacuo* without the addition of acid, because to all appearances certain active constituents of the serum, acting like acids, expel carbon dioxide from the simple carbonate. The quantity of carbon dioxide not expelled from dog-serum by vacuum alone without the addition of acid amounts to 4.9 to 9.3 vols. per cent, according to the determinations of PFLÜGER.[1]

From the occurrence of simple alkali carbonates in the blood-serum it naturally follows that a part of the loosely combined carbon dioxide of the serum which can be pumped out must exist as bicarbonate. The occurrence of this combination in the blood-serum has also been directly shown. In experiments with the pump, as well as in absorption experiments, the serum behaves in other ways differently from a solution of bicarbonate, or carbonate of a corresponding concentration; and the action of the loosely combined carbon dioxide in the serum can be explained only by the occurrence of bicarbonate in the serum. By means of a vacuum, the serum always allows much more than one-half of the carbon dioxide to be expelled and it follows from this that in the pumping out

[1] E. Pflüger's Ueber die Kohlensäure des Blutes, Bonn, 1864, 11. Cited from Zuntz in Hermann's Handbuch, 65.

not only may a dissociation of the bicarbonate take place, but also a conversion of the double sodium carbonate into a simple salt. As we know of no other carbon-dioxide combination, besides the bicarbonate, in the serum, from which the carbon dioxide can be set free by simple dissociation *in vacuo*, it must be assumed that the serum contains other weak acids, in addition to the carbon dioxide, which contend with it for the alkalies, and which expel the carbon dioxide from simple carbonates *in vacuo*. The carbon dioxide which is expelled by means of the pump, and which, without regard to the quantity merely absorbed, is generally designated as "carbon dioxide in loose chemical combination," is thus only obtained in part in dissociable loose combinations; in part it originates from the simple carbonates, from which it is expelled, *in vacuo*, by other weak acids.

These weak acids are thought to be in part phosphoric acid and in part globulins. The importance of the alkali phosphates in the carbon dioxide combination has been shown by the investigations of FERNET; but the quantity of these salts in the serum is, at least in certain kinds of blood, for example, in ox-serum, so small that it can hardly be of importance. In regard to the globulins, SETSCHENOW is of the opinion that they do not act as acids themselves, but form a combination with carbon dioxide, producing carboglobulinic acid, which unites with the alkali. According to SERTOLI,[1] whose views have found a supporter in TORUP, the globulins themselves are the acids which are combined with the alkali of the blood-serum. In both cases the globulins would form, directly or indirectly, that chief constituent of the plasma or of the blood-serum which, according to the law of mass action, contends with the carbon dioxide for the alkalies. By a greater partial pressure of the carbon dioxide the latter deprives the globulin alkali of a part of its alkali, and bicarbonate is formed; by low partial pressure carbon dioxide is set free and it is abstracted from the bicarbonate by the globulin alkali. It must also be added that the above-mentioned carboglobulinic acid can perhaps be considered as a dissociable combination of carbonic acid and protein.

The assumption that the proteins of the blood are bodies active in combining with the carbon dioxide has received some support from the investigations of SIEGFRIED[2] on the combination of carbon dioxide with amphoteric amino bodies. SIEGFRIED has found that amino-acids combine with carbon dioxide, thereby being converted into carbamino-

[1] Hoppe-Seyler, Med. chem. Untersuch., 350.
[2] Zeitschr. f. physiol. Chem., 44 and 46.

acids—glycocoll for example, into carbamino acetic acid, $CH_2(COOH)—NH—COOH$,

and that the carbon dioxide can be readily split off from these compounds. The peptones and serum proteins in the presence of calcium hydroxide may also act in the same manner as amino-acids. Protein carbamino-acids are formed, and the possibility of such a binding of carbon dioxide must also be considered.

In the foregoing it has been assumed that the alkali is the most essential and important constituent of the blood-serum, as well as of the blood in general, in uniting with the carbon dioxide. The fact that the quantity of carbon dioxide in the blood greatly diminishes with a decrease in the quantity of alkali strengthens this assumption. Such a condition is found, for example, after poisoning with mineral acids. Thus WALTER found only 2–3 vols. per cent carbon dioxide in the blood of rabbits into whose stomachs hydrochloric acid had been introduced. In the comatose state of diabetes mellitus the alkali of the blood seems to be in great part saturated with acid combinations, β-oxybutyric acid (STADELMANN, MINKOWSKI), and MINKOWSKI [1] found only 3.3 vols. per cent carbon dioxide in the blood in diabetic coma.

Gases of the Lymph and Secretions.

The gases of the lymph are the same as in the blood-serum, and the lymph stands close to the blood-serum in regard to the quantity of the various gases, as well as to the kind of carbon-dioxide combination. The investigations of DAENHARDT and HENSEN [2] on the gases of human lymph are at hand, but it still remains a question whether the lymph investigated was quite normal. The gases of normal dog-lymph were first investigated by HAMMARSTEN.[3] This gas contained traces of oxygen and consisted of 37.4–53.1 per cent CO_2 and 1.6 per cent N at 0° C. and 760 mm. Hg pressure. About one-half of the carbon dioxide was in firm chemical combination. The quantity was greater than in the serum from arterial blood, but smaller than from venous blood.

The remarkable observation of BUCHNER, that the lymph collected after asphyxiation is poorer in carbon dioxide than that of the breathing

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[1] Walter, Arch. f. exp. Path. u. Pharm., 7; Stadelmann, *ibid.*, 17; Minkowski Mittheil a. d. med. Klinik in Königsberg, 1888.

[2] Virchow's Arch., 37.

[3] Ber. d. k. sächs. Gesellsch. d. Wissensch., math.-phys. Klasse, 23.

animal, is explained by ZUNTZ [1] by the formation of acid in the tissues, and especially in the lymphatic glands, immediately after death, and this acid in part decomposes the alkali carbonates of the lymph.

The secretions, with the exception of the saliva, in which PFLÜGER and KÜLZ found respectively 0.6 per cent and 1 per cent oxygen, are almost free from oxygen. The quantity of nitrogen is the same as in blood, and the chief mass of the gases consists of carbon dioxide. The quantity of this gas is chiefly dependent upon the reaction, i.e., upon the quantity of alkali. This follows from the analyses of PFLÜGER. He found 19 per cent carbon dioxide removable by the air-pump and 54 per cent firmly combined carbon dioxide in a strongly alkaline bile, but, on the contrary, 6.6 per cent carbon dioxide removable by the air-pump and 0.8 per cent firmly combined carbon dioxide in a neutral bile. Alkaline saliva is also very rich in carbon dioxide. As average for two analyses made by PFLÜGER of the submaxillary saliva of a dog we have 27.5 per cent carbon dioxide removable by the air-pump and 47.4 per cent chemically combined carbon dioxide, making a total of 74.9 per cent. KÜLZ [2] found a maximum of 65.78 per cent carbon dioxide for the parotid saliva, of which 3.31 per cent was removable by the air-pump and 62.7 per cent was firmly combined. From these and other reports as to the quantity of carbon dioxide removable by the air-pump and chemically combined in the alkaline secretions it follows that bodies occur in them, although not in appreciable quantities, which are analogous to the protein bodies of the blood-serum and which act like weak acids.

The acid or at any rate non-alkaline secretions, urine and milk, contain, on the contrary, considerably less carbon dioxide, which is almost all removable by the air-pump, and a part seems to be loosely combined with the sodium phosphate. The figures found by PFLÜGER for the total quantity of carbon dioxide in milk and urine are 10 and 18.1–19.7 per cent respectively.

EWALD [3] made investigations on the quantity of gas in pathological transudates. He found only traces, or at least only very insignificant quantities of oxygen in these fluids. The quantity of nitrogen was about the same as in blood; that of carbon dioxide was greater than in the lymph (of dogs), and in certain cases even greater than in the blood after asphyxiation (dog's blood). The tension of the carbon dioxide was greater than in venous blood. In exudates the quantity of carbon dioxide, especially that firmly combined, increases with the age of the

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[1] Buchner, Arbeiten aus der physiol. Anstalt zu Leipzig, 1876; Zuntz, l. c., 85.

[2] Pflüger, Pflüger's Arch., 1 and 2; Külz, Zeitschr. f. Biologie, 23. It seems as if Külz's results were not calculated at 760 millimeters Hg. but rather at 1 meter.

[3] C. A. Ewald, Arch. f. (Anat. u.) Physiol., 1873 and 1876.

fluid, while, on the contrary, the total quantity of carbon dioxide, and especially the quantity firmly combined, decreases with the quantity of pus-corpuscles.

II. THE EXCHANGE OF GAS BETWEEN THE BLOOD, ON THE ONE HAND, AND PULMONARY AIR AND THE TISSUES, ON THE OTHER.

In Chapter I (page 42) it was stated that we are to-day of the opinion, derived especially from the researches of PFLÜGER and his pupils, that the oxidations of the animal body do not take place in the fluids and juices, but are connected with the form-elements and tissues. It is nevertheless true that oxidations take place in the blood itself, although, only to a slight extent; but these oxidations depend, it seems, upon the form-elements of the blood, hence it does not contradict the above statement that the oxidations exclusively occur in the cells and chiefly in the tissues.

The gaseous exchange in the tissues, which has been designated internal respiration, consists chiefly in that the oxygen passes from the blood in the capillaries to the tissues, while the great bulk of the carbon dioxide of the tissues originates therein and passes into the blood of the capillaries. The exchange of gas in the lungs, which is called external respiration, consists, as is seen by a comparison of the inspired and expired air, in the blood taking oxygen from the air in the lungs and giving off carbon dioxide. This does not exclude the fact that in the lungs, as in every other tissue, an internal respiration takes place, namely, a combustion with a consumption of oxygen and formation of carbon dioxide. According to BOHR and HENRIQUES[1] the lungs take a variable but sometimes a very important part in the total metabolism. This part, which on an average is 33 per cent, but may even rise above 60 per cent of the total metabolism, depends, these experimenters say, upon the fact that the intermediary metabolic products formed in the tissues are burnt in the lungs. It is also in part represented by the specific work of the lungs.

What kind of processes take part in this double exchange of gas? Is the gaseous exchange simply the result of an unequal tension of the blood on one side and the air in the lungs or tissues on the other? Do the gases pass from a place of higher pressure to one of a lower, according to the laws of diffusion, or are other forces and processes active?

These questions are closely related to that of the tension of the oxygen and carbon dioxide in the blood and in the air of the lungs and tissues.

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[1] Centralbl. f. Physiol., 6, and Maly's Jahresber., 27.

Oxygen occurs in the blood in a disproportionately large part as oxyhæmoglobin, and the law of the dissociation of oxyhæmoglobin is of fundamental importance in the study of the tension of the oxygen in the blood.

Attempts have been made to prove this law by investigations on pure solutions of hæmoglobin, and HÜFNER [1] has made very careful and important determinations on such solutions. Recent investigations of BOHR [2] and his pupils, as well as of LOEWY and ZUNTZ,[3] have shown that the conditions in the blood are different from a pure hæmoglobin solution, which, in part, may be due to a change in the hæmoglobin brought about in its preparation. A hæmoglobin solution in which alcohol is used in preparing it, combines more firmly with oxygen than the blood, and the dissociation tension of the oxygen is greater in blood than in such a hæmoglobin solution.

The oxygen tension may be variable, as LOEWY [4] has shown, with different individuals, and it is not the same in the blood of different animals with the same oxygen pressure; for example, it is less in cat's blood than in the dog, horse and human blood. The temperature is also of great importance, as the dissociation tension increases with a rise in temperature, and with the same pressure the blood combines with less oxygen at a high temperature than at a low temperature. The influence of the concentration of the hæmoglobin manifests itself in that in dilute solutions the oxygen is more firmly combined (HÜFNER, LOEWY and ZUNTZ, BOHR) and that consequently blood made laky with water has a lower dissociation tension and a firmer binding of the oxygen than undiluted blood.

Of especial interest is the finding of BOHR, HASSELBALCH and KROGH [5] that the CO_2 present also influences the oxygen taken up, in that as the carbon dioxide tension (also within physiological limits) increases the oxygen taken up diminishes. The laws of oxygen absorption must be determined by determinations upon blood itself, at the same time observing the temperature and the carbon dioxide tension. A series of determinations made by KROGH [6] upon horse's blood at 38°, and a constant carbon dioxide tension, is given below. In calculating the results in column 4 the quantity of oxygen chemically combined at 150 mm. oxygen pressure is equal to 100.

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[1] Arch. f. (Anat. u.) Physiol., 1890 and 1894.
[2] See Nagel's Handbuch, and Krogh, Skand. Arch. f. Physiol., **16**.
[3] Arch. f. (Anat. u.) Physiol., 1904.
[4] *Ibid.*
[5] Centralbl. f. Physiol., **17**, and Skand. Arch. f. Physiol., **16**.
[6] Skand. Arch. f. Physiol., **16**.

Oxygen tension in mm.	In 100 cc. blood: Chemically combined oxygen	In 100 cc. blood: Oxygen dissolved in plasma	Oxygen taken up: Per cent chemically combined	Oxygen taken up: Dissolved in 100 cc. plasma
10	6.0	0.020	30.0	0.030
20	12.9	0.041	64.7	0.061
30	16.3	0.061	81.6	0.091
40	18.1	0.081	90.4	0.121
50	19.1	0.101	95.4	0.152
60	19.5	0.121	97.6	0.182
70	19.8	0.141	98.8	0.212
80	19.9	0.162	99.5	0.243
90	19.95	0.182	99.8	0.273
150	20.00	0.303	100.0	0.455

From the above table we see that even with an oxygen tension which amounts to only one-half of the oxygen pressure in the air, the hæmoglobin is saturated in greatest part with oxygen. The dissociation is hence at 70–80 mm. pressure only slightly more than with a pressure of 150 mm. and indeed even with as low a pressure as 40–30 mm., still 90–80 per cent of the entire quantity of oxygen taken up chemically at 150 mm. is combined with the hæmoglobin.

From these and other observations it follows that the oxygen partial pressure may sink to one-half of that existing in the atmospheric air without markedly influencing the oxygen content of the blood. This also coincides with the experience of FRÄNKEL and GEPPERT[1] on the action of low air pressures upon the oxygen content of the blood of dogs. With an air pressure of 410 mm. Hg, they found that the oxygen content of arterial blood was normal. With an air pressure of 378–365 mm. it was slightly diminished, and only on reducing the pressure to 300 mm. was a noticeable decrease observed. A. LOEWY[2] found that the lowest oxygen pressure of the alveolar air wherein the exchange of material can go on normally both qualitatively and quantitatively, is equal to 30 mm. Hg.

In regard to the above-mentioned action of low air pressure it must be remarked that the alveolar oxygen tension is regulated by changes in the respiration, so that with great diminution in the quantity of oxygen of the inspired air, the alveolar air contains the same quantity of oxygen as with a higher oxygen partial pressure of the inspired air (LOEWY). For example, LOEWY found the same quantity of oxygen, namely, 6.1 per cent, in the alveolar air with 16 and with 10.5 per cent oxygen in the inspired air, because the respiration in the latter case was 8.5 liters per minute against only 4.9 liters in the first case.

It may be concluded from the large quantity of oxygen or oxyhæmo-

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[1] Ueber die Wirkungen der verdünnten Luft auf den Organismus, Berlin, 1883.

[2] A. Loewy, Untersuch. über die Respiration und Zirculation, etc., Berlin, 1895; also Centralbl. f. Physiol., 13, 449, and Arch. f. (Anat. u.) Physiol., 1900.

globin in the arterial blood that the tension of the oxygen in the arterial blood must be relatively higher. This is substantiated by the earlier observations of BERT and HÜFNER, as well as by the determinations of HERTER, FRÉDERICQ and others,[1] using aerotonometric methods, which will be mentioned below in connection with the carbon dioxide tension. HERTER found the oxygen tension in the arterial blood of dogs to be equal, on an average, to a pressure of 78.7 mm. Hg and FRÉDERICQ, by a better method, found that it was equal to 91–99 mm. Hg.

The oxygen tension of the venous blood of dogs has been found by aerotonometric means to be equal to 20.6–27.7 mm. (STRASSBURG, FALLOISE), and by means of the lung-catheter (see below) equal to 25.5–27 mm. (WOLFBERG, NUSSBAUM). For human venous blood LOEWY and v. SCHRÖTTER[2] found an average of 37.68 mm. Concerning the question as to the mechanism of taking up oxygen in the lungs these figures are of less interest than the oxygen tension in the arterial blood, that is, that which has left the lungs, whose tension is estimated as 90 to about 100 mm. Hg as given above.

These results do not coincide with the investigations of BOHR,[3] who in many cases obtained essentially higher figures for the oxygen tension in arterial blood.

He experimented on dogs, allowing the blood, whose coagulation had been prevented by the injection of peptone solution or infusion of the leech, to flow from one bisected carotid to the other, or from the femoral artery to the femoral vein, through an apparatus called by him an hæmataërometer. The apparatus, which is a modification of LUDWIG'S rheometer (*stromuhr*), allowed, according to BOHR, of a complete interchange between the gases of the blood circulating through the apparatus and a quantity of gas whose composition was known at the beginning of the experiment and inclosed in the apparatus. The mixture of gases was analyzed after an equalization of the gases by diffusion. In this way the tension of the oxygen and carbon dioxide in the circulating arterial blood was determined. During the experiment the composition of the inspired and expired air was also determined, the number of inspirations noted, and the extent of respiratory exchange of gas measured. To be able to make a comparison between the gas tension in the blood and in an expired air whose composition was closer to the unknown composition of the alveolar air than the ordinary expired air, the composition of the expired air at the moment it passed the bifurcation of the trachea was ascertained by special calculation. The tension of the gases in this " bifurcated air " could be compared with the tension of the gases of the blood, and in such a way that the comparison took place simultaneously. Recently

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[1] Pert, La pression barometrique, Paris, 1878; Herter, Zeitschr. f. physiol. Chem. **3**; Hüfner, l. c.; Frédericq, Centralbl. f. Physiol., **7**, and Travaux der laborat. de l'inst. de physiol. de Liège, **5**, 1896.

[2] Strassburg, Pflüger's Arch., **6**; Falloise, Bull. Acad. Roy. Belg., 1902. Wolfberg, Pflüger's Arch., **4** and **6**; Nussbaum, *ibid.*, *l*; Loewy and v. Schrötter, cited by Loewy in Oppenheimer's Handb., **4**, 76.

[3] Skand. Arch. f. Physiol., **2**, and Nagel's Handbuch der Physiologie.

KROGH [1] constructed an apparatus, called by him *microtonometer*, to be used for the same purpose.

BOHR found remarkably high results for the oxygen tension in arterial blood in this series of experiments. They varied between 101 and 144 mm. Hg pressure. In eight out of nine experiments on the breathing of atmospheric air, and in four out of five experiments on breathing air containing carbon dioxide, the oxygen tension in the arterial blood was higher than the "bifurcated air." The greatest difference, where the oxygen tension was higher in the blood than in the air of the lungs, was 38 mm. Hg.

HÜFNER and FRÉDERICQ [2] have made the objection to BOHR'S experiments and views that a perfect equilibrium had probably not been attained between the air in the apparatus and the gases of the blood. FRÉDERICQ, by new experiments, presents strong objections to the acceptance of BOHR'S findings, while on the other hand BOHR not only defends his experiments, but also finds errors in the experiments of his opponents, while HALDANE and SMITH'S [3] experiments, making use of an entirely different principle, tend to corroborate the high results attained by BOHR.

HALDANE'S method is as follows: The individual experimented upon is allowed to inspire air containing an exactly known but small quantity of carbon monoxide (0.045-0.06 per cent), until no further absorption of carbon monoxide takes place and the percentage saturation of the hæmoglobin in the arterial blood with carbon monoxide has become constant, as shown by a special titration method. This percentage saturation is dependent upon the relation between the tension of the oxygen in the blood and the tension of the carbon monoxide, as known from the composition of the inspired air. When this last and the percentage saturation with carbon monoxide and oxygen are known the oxygen tension in the blood can be easily calculated.

According to this method HALDANE and SMITH found still higher figures than BOHR for the oxygen tension in the blood, and they calculated the average tension of the oxygen in human arterial blood to be equal to 293 mm. Hg.

Based upon the experiments of A. and M. KROGH, which will be discussed below (page 864) A. KROGH [4] has presented objections to the experiments of HALDANE and SMITH.

Let us now compare the figures for the oxygen tension of the arterial blood as found by various investigators with the tension of the oxygen in the air of the lungs.

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[1] Skand. Arch. f. Physiol., **20**.

[2] Hüfner, Arch. f. (Anat. u.) Physiol., 1890; Frédericq, Centralbl. f. Physiol., **7**, and Traveaux du laboratorie de l'institute de physiologie de Liège, **5**, 1896.

[3] Haldane, Journ. of Physiol., 18; Haldane and Smith, *ibid.*, **20**.

[4] Skand. Arch. f. Physiol., **23**, 217, 253.

Numerous investigations as to the composition of the inspired atmospheric air as well as the expired air are at hand, and it can be said that these two kinds of air at 0° C. and a pressure of 760 mm. Hg have the following average composition in volume per cent.

	Oxygen	Nitrogen (and argon)	Carbon Dioxide
Atmospheric air	20.96	79.02	0.03
Expired air	16.03	79.59	4.38

The partial pressure of the oxygen of the atmospheric air corresponds at a normal barometric pressure of 760 mm. to a pressure of 150 mm. Hg. The loss of oxygen which the inspired air suffers in respiration amounts to about 4.93 per cent, while the expired air contains about one hundred times as much carbon dioxide as the inspired air.

The expired air is therefore a mixture of alveolar air with the residue of inspired air remaining in the air-passages; hence in the study of the gaseous exchange in the lungs the alveolar air must first be considered. There exists no direct determination of the composition of the alveolar air in man, but only approximate calculations. From the average results found by VIERORDT in normal respiration for the carbon dioxide in the expired air, 4.63 per cent, ZUNTZ [1] has calculated the probable quantity of carbon dioxide in the alveolar air as equal to 5.44 per cent. If we start from this value, with the assumption that the quantity of nitrogen in the alveolar air does not essentially differ from the expired air, and admit that the quantity of oxygen in the alveolar air is 6 per cent less than the inspired air, it will be seen that the alveolar air contains 15 per cent oxygen. As the total pressure of the air of the lungs after deducting the aqueous tension of about 50 mm. can be calculated as about 710 mm. the partial pressure of the oxgyen in man can be put at about 106 mm. and that of the carbon dioxide as about 45 mm.

Based upon several respiration experiments upon different persons, LOEWY has been able to calculate the composition of the alveolar air of human beings almost at the atmospheric pressure, from the composition of the expired air and the depth of inspiration and expiration, taking into consideration the air in the upper air-passages. He obtained results which varied between 101 and 105 mm. Hg for the oxygen tension and between 32–42 mm. for the carbon dioxide tension.

The alveolar oxygen tension in dogs can be calculated from the carbon dioxide content of the alveolar air and is also found to be above 100 mm. Hg.

If the oxygen partial pressure in the alveoli is put at about 105

[1] See Zuntz, l. c., Hermann's Handbuch, 105 and 106.

mm. Hg and we compare this with the highest results obtained for the oxygen tension of the arterial blood as determined by tonometric means, we find that the taking up of oxygen in the lungs can be simply explained according to physical laws as a diffusion process. The conditions are quite different if we start with the high-tension results of BOHR, 101–144 mm. Hg, or the still higher results of HALDANE and SMITH. The oxygen tension in the blood is, in many cases, according to these latter authors, always higher than the tension in the lungs, as average for various races of animals. In these cases the passage of oxygen from the lungs to the blood cannot be explained simply by a diffusion. We must therefore with BOHR, accept a special activity of the lungs, and according to, him a secretory activity of the lungs also exists besides diffusion. In his most recent work BOHR [1] presents the view that the specific action of the lungs essentially consists in maintaining a necessary difference in pressure for the diffusion. Nevertheless besides this a secretory process is necessary, especially for the taking up of oxygen. Based upon newer measurements DOUGLAS and HALDANE [2] also advocate the view that the taking up of oxygen can be brought about by diffusion alone, but that with the existing lack of oxygen in the tissues an active secretion of oxygen takes place in the lungs.

By means of a tonometer described by A. KROGH, he and M. KROGH have compared the oxygen tension in the arterial blood with that in the alveolar air. In these experiments the tension in the blood was always found lower than in the alveolar air. From this A. KROGH [3] concludes that the exchange of gas in the lungs is chiefly brought about by diffusion. FRÉDERICQ [4] has recently arrived at the same view by his experiments on the respiratory exchange of gas in aquatic animals.

As reports on the taking up of oxygen are conflicting so also are those on the giving up of carbon dioxide.

The tension of the carbon dioxide in the blood has been determined in different ways by PFLÜGER and his pupils WOLFFBERG, STRASSBURG, and NUSSBAUM.[5]

According to the aërotonometric method the blood is allowed to flow directly from the artery or vein through a glass tube which contains a gas mixture of a known composition. If the tension of the carbon dioxide in the blood is greater than the gas mixture, then the blood gives up carbon dioxide, while in the reverse case it takes up carbon dioxide from the gas mixture. The analysis of the gas

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[1] Centralbl. f. Physiol. **23**, 274; Skand. Arch. f. Physiol., **22**, 221 (1909).

[2] Skand. Arch. f. Physiol., **25**, 169 (1911); Proc. Roy. Soc., 1911; see also Journ. of Physiol., **44**, 305 (1912).

[3] Skand. Arch. f. Physiol., **20**, 263; **23**, 179, 200, 213, (1910).

[4] Arch. intern. de Physiol., **10**, 391 (1911).

[5] Wolffberg, Pflüger's Arch., **6**; Strassburg, *ibid.*; Nussbaum, *ibid.*, **7**.

mixture after passing the blood through it will also decide if the tension of the carbon dioxide in the blood is greater or less than in the gas mixture; and by a sufficiently great number of determinations, especially when the quantity of carbon dioxide of the gas mixture corresponds as closely as possible, in the beginning, to the probable tension of this gas in the blood, we may learn the tension of the carbon dioxide in the blood. As above mentioned the oxygen tension can be determined by the same method.

According to this method the carbon-dioxide tension of the arterial blood is on an average 2.8 per cent of an atmosphere,[1] corresponding to a pressure of 20 mm. mercury (STRASSBURG). In the blood from the pulmonary aveoli NUSSBAUM found a carbon-dioxide tension of 3.81 per cent of an atmosphere, corresponding to a pressure of 27 mm. mercury. STRASSBURG, who experimented in non-tracheotomized dogs in which the ventilation of the lungs was less active and therefore the carbon dioxide was removed from the blood with less readiness, found in the venous blood of the heart, a carbon-dioxide tension of 5.4 per cent of an atmosphere, corresponding to a partial pressure of 38.3 mm. mercury.

Another method, which was first used by PFLÜGER and his pupils WOLFFBERG and NUSSBAUM, depends upon excluding a part of the lungs be means of the *lung catheter* .

The principle of this method is as follows: By the introduction of a catheter, of a special construction, into a branch of a bronchus the corresponding lobe of the lung may be hermetically sealed, while in the other lobes of the same lung, and in the other lung, the ventilation remains unchanged, so that no accumulation of carbon dioxide takes place in the blood. When the cutting off lasts so long that a complete equalization between the gases of the blood and the retained air of the lungs is assumed, a sample of this air of the lungs is removed by means of the catheter and analyzed.

When a complete exchange between the gases of the inclosed part of the lungs and the gases of the circulating venous blood has taken place, the tension of the gases in this part of the lungs can be considered as a measure for the gas tension in the venous blood, if we admit that the gas exchange is due only to physical forces. In their experiments WOLFFBERG and NUSSBAUM found only 3.6 per cent CO_2 in the air taken out with the catheter. NUSSBAUM also determined the carbon-dioxide tension in the blood from the right heart in a case simultaneous with the catheterization of the lungs. He found almost identical results, namely, a carbon-dioxide tension of 3.84 per cent and 3.81 per cent of an atmosphere, which also shows that complete equalization between the gases of the blood and lungs in the inclosed parts of the lungs had taken

[1] Here and in the following discussion we mean by atmospheric pressure the pressure in the lungs after subtracting the aqueous vapor tension (about 50 mm.), namely, 760 − 50 = 710 mm. mercury pressure.

place. The method of catheterizing the lungs is, as shown by LOEWY and v. SCHRÖTTER,[1] also applicable to man, and they found that the carbon dioxide tension of human venous blood was equal to 6 per cent of the atmospheric pressure in the lungs = 42.6 mm. Hg, while according to LOEWY'S calculations the carbon dioxide tension in the respired lung aveoli varied between 31.8 and 41.8 mm. Hg with an average of 37.3 mm. Hg for eleven cases.

According to these investigations the giving up of carbon dioxide may also be explained by physical laws; but BOHR, in his experiments above mentioned (page 861), has arrived at other results in regard to the carbon-dioxide tension. In eleven experiments with inhalation of atmospheric air the carbon-dioxide tension in the arterial blood varied from 0 to 38 mm. Hg, and in five experiments with inhalation of air containing carbon dioxide, from 0.9 to 57.8 mm. Hg. A comparison of the carbon-dioxide tension in the blood with the bifurcated air gave in several cases a greater carbon-dioxide pressure in the air of the lungs than in the blood, and as maximum this difference amounted to 17.2 mm. in favor of the air of the lungs in the experiments with inhalation of atmospheric air. As the aveolar air is richer in carbon dioxide than the bifurcated air this experiment unquestionably proves, according to BOHR, that the carbon dioxide has migrated against the high pressure.

In opposition to these investigations, FRÉDERICQ,[2] in his above-mentioned experiments, obtained the same figures for the carbon-dioxide tension in arterial peptone blood as PFLÜGER and his pupils found for normal blood. WEISGERBER,[3] in FRÉDERICQ'S laboratory, has made experiments with animals which respired air rich in carbon dioxide, and these experiments confirm PFLÜGER'S theory of respiration. Recently FALLOISE has made determinations of the carbon-dioxide tension of venous blood by means of FRÉDERICQ'S aërotonometer. The carbon-dioxide tension was found to equal 6 per cent of an atmosphere, hence somewhat higher than the results found by PFLÜGER'S pupils. To these investigations BOHR has presented strong objections; he has demonstrated the principles for the construction of the tonometer, and claims that the earlier experiments with the tonometer are not conclusive, as a complete equilibrium of the gas tension was not attained.

A certain importance has been ascribed to oxygen in regard to the elimination of carbon dioxide in the lungs, in that it has an expelling action on the carbon dioxide from its combinations in the blood. This theory, first advanced by HOLMGREN, has recently found an advocate

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[1] l. c., footnote 2, page 861.

[2] See footnote 1, page 861.

[3] Centralbl. f. Physiol., 10, 482; Falloise, see Maly's Jahresber., 32.

in WERIGO. Still ZUNTZ has presented weighty objections to WERIGO'S experiments, and BOHR [1] has later also shown that we have no positive basis for the above assumption.

The conditions as to the elimination of carbon dioxide in the lungs are not quite clear. On the one hand we have advocates of the view that the gas exchange takes place simply according to physical laws and is chiefly considered as a diffusion process. According to the former views of BOHR,[2] which he has supported by recent experiments, a diffusion does take place, but the lung is a gland which has the power of secreting gases, and the gas exchange in the lungs is essentially a secretory process. In his most recent work BOHR,[3] after a thorough criticism of the methods used in the measurement of the lung diffusion and based upon a new calculation of the extent of diffusion, has come to the conclusion that the specific activity of the lungs consists in that a difference in pressure necessary for the diffusion is brought about.

That a true secretion of gases occurs in animals follows from the composition and behavior of the gases in the swimming-bladder of fishes. These gases consist of oxygen and nitrogen with only small quantities of carbon dioxide. In fishes which do not live at any great depth the quantity of oxygen is ordinarily as high as in the atmosphere, while fishes which live at great depths may, according to BIOT and others, contain considerably more oxygen and even above 80 per cent. MOREAU has also found that after emptying the swimming-bladder by means of a trocar, new air collected after a time, and this air was richer in oxygen than the atmospheric air, and contained even 85 per cent oxygen. BOHR, who has proven and confirmed these statements, also found that this accumulation is under the influence of the nervous system, because on the section of certain branches of the pneumogastric nerve it is discontinued. It is beyond dispute that there is here a secretion and not a diffusion of oxygen. Recently JAEGER [4] has given a further explanation as to the secretory activity of the swimming-bladder.

From what has been said above (page 858) in regard to the internal respiration, one can conclude that it consists chiefly that in the capillaries the oxygen passes from the blood into the tissues, while the carbon dioxide passes from the tissues into the blood.

The assertion of ESTOR and SAINT PIERRE that the quantity of oxygen in the blood of the arteries decreases with the remoteness from the heart has been shown to be incorrect by PFLÜGER,[5] and the oxygen tension in blood on entering the capillaries must be higher. The oxygen tension

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[1] Holmgren, Wien. Sitzungsber., 48; Werigo, Pflüger's Arch., 51 and 52; Zuntz, *ibid.*, 52; Bohr, see Nagel's Handbuch der Physiologie.

[2] Centralbl. f. Physiol., 21, 367.

[3] *Ibid.*, 23, 374; Skand. Arch. f. Physiol., 22, 221 (1909).

[4] Biot, see Hermann's Handbuch d. Physiol., 4, Thl. 2, 151; Moreau, Compt. Rend., 57; Bohr, Journ. of Physiol., 15. See also Hüfner, Arch. f. (Anat. u.) Physiol., 1892; Jaeger, Pflüger's Arch., 94.

[5] Estor and Saint Pierre with Pflüger in Pflüger's Arch., 1.

of the plasma is of importance in the giving up of oxygen to the tissues, as the blood corpuscles contain a supply of oxygen only sufficient to replace that removed from the plasma by the tissue. This quantity of oxygen, which is dissolved in the plasma and at the disposal of the tissues, is dependent upon the oxygen tension in the blood and only indirectly dependent upon the total quantity of oxygen in the blood. As this tissue is almost or entirely free from oxygen, a considerable difference in regard to the oxygen pressure must exist between the blood and the tissues. The possibility that this difference in pressure is sufficient to supply the tissues with the necessary quantity of oxygen is hardly to be doubted.

The animal body, it seems, also has the command over means of regulating and varying the oxygen tension, and such a means is the carbon dioxide produced in the tissue which, according to BOHR, HASSELBALCH, and KROGH,[1] raises the oxygen tension. This is of special importance when the tension of the oxygen in the blood of the capillaries is very low; then the ability of the carbon dioxide to raise the dissociation tension of the oxyhæmoglobin comes into play, especially with low oxygen tension. Another regulating moment is, BOHR claims, the *specific oxygen capacity* of the blood, which means the relation of the maximum oxygen combination to the quantity of iron of the blood or the hæmoglobin solution.

In regard to the carbon-dioxide tension in the tissue it must be assumed *a priori* that it is higher than in the blood. This is found to be true. STRASSBURG[2] found in the urine of dogs and in the bile a carbon-dioxide tension of 9 per cent and 7 per cent of an atmosphere, respectively. The same experimenter has, further, injected atmospheric air into a ligatured portion of the intestine of a living dog and analyzed the air taken out after some time. He found a carbon-dioxide tension of 7.7 per cent of an atmosphere. The carbon-dioxide tension in the tissues is considerably greater than in the venous blood, and there is no opposition to the view that the carbon dioxide simply diffuses from the tissues into the blood according to the law of diffusion.

Several methods have been suggested for the study of the quantitative relation of the respiratory exchange of gas. The reader must be referred to other text-books for details as to these methods, and we will here mention only the chief features of the most important methods. It must also be remarked, in regard to these methods, that those of REGNAULT and REISET and of PETTENKOFER, determine the total gas exchange, and indeed for a long time, while the other three methods determine the respiratory gas exchange alone, and this only for a short time.

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[1] Centralbl. f. Physiol., **17**, and Skand. Arch. f. Physiol., **16**.

[2] Pflüger's Arch., **6**.

REGNAULT and REISET'S *Method.* According to this method the animal or person experimented upon is allowed to respire in an inclosed space. The carbon dioxide is removed from the air, as it forms, by strong caustic alkali, from which the quantity may be determined, while the oxygen is replaced continually in exactly measured quantities. This method, which also makes possible a direct determination of the oxygen used as well as the carbon dioxide produced, has since been modified by other investigators, such as PFLÜGER and his pupils, SEEGEN and NOWAK, and HOPPE-SEYLER, ROSENTHAL and OPPENHEIMER and especially by ATWATER and BENEDICT.[1]

PETTENKOFER'S *Method.* According to this method the individual to be experimented upon breathes in a room through which a current of atmospheric air is passed. The quantity of air passed through is carefully measured. As it is impossible to analyze all the air made to pass through the chamber, a small fraction of this air is diverted into a branch line during the entire experiment, carefully measured, and the quantity of carbon dioxide and water determined. From the composition of this air the quantity of water and carbon dioxide contained in the large quantity of air made to pass through the chamber can be calculated. The consumption of oxygen cannot be directly determined in this method, but may be calculated indirectly by difference, which is a defect in this method. The large respiration apparatus of SONDÉN and TIGERSTEDT as well as of ATWATER and ROSA [2] are based upon this principle.

SPECK'S *Method.*[3] For briefer experiments on man SPECK used the following: He breathes through a mouthpiece with two valves, closing the nose with a clamp, into two spirometer-receivers, where the gas-volume can be read off very accurately. The air from one of the spirometers is inhaled through one valve and the expired air passes through the other into the other spirometer. By means of a rubber tube connected with the expiration-tube an accurately measured part of the expired air may be passed into an absorption-tube and analyzed.

ZUNTZ and GEPPERT'S *Method.*[4] This method, which has been improved by ZUNTZ and his pupils from time to time, consists in the following: The individual being experimented upon inspires pure atmospheric air through a very wide feed-pipe leading from the open air, the inspired and the expired air being separated by two valves (human subjects breathe with closed nose by means of a soft-rubber mouthpiece, animals through an air-tight tracheal canula). The volume of the expired air is measured by a gas-meter and an aliquot part of this air collected and the quantity of carbon dioxide and oxygen determined. As the composition of the atmospheric air can be considered as constant within a certain limit, the production of carbon dioxide as well as the consumption of oxygen may be readily calculated (see the works of ZUNTZ and his pupils).

HANRIOT and RICHET'S *Method*[5] is characterized by its simplicity. These investigators allow the total air to pass through three gasometers, one after the other. The first measures the inspired air, whose composition is known. The second gasometer measures the expired air, and the third the quantity of the

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[1] See Zuntz in Hermann's Handbuch, 4, Thl. 2, and Hoppe-Seyler, Zeitschr. f. physiol. Chem., 19; Rosenthal, Arch. f. (Anat. u.) Physiol., 1902; Zuntz and Oppenheimer, Arch. f. (Anat. u.) Physiol., 1905, and Bioch. Zeitschr., 14; Atwater and Benedict, Bull. Dept. of Agriculture, Washington, 69, 109, and 136. See also Krogh, Wien. Sitz. Ber., 115, III., and Skand. Arch. f. Physiol., 18.

[2] Pettenkofer's method; see Zuntz, l. c.; Sondén and Tigerstedt, Skand. Arch. f. Physiol., 6; Atwater and Rosa, Bull. of Dept. of Agriculture, 62. Washington.

[3] Speck, Physiologie des menschlichen Atmens. Leipzig, 1892.

[4] Pflüger's Arch., 42. See also Magnus-Levy in Pflüger's Arch., 55, 10, in which the work of Zuntz and his pupils is cited.

[5] Compt. Rend., 104.

expired air after the carbon dioxide has been removed by a suitable apparatus. The quantity of carbon dioxide produced and the oxygen consumed can be readily calculated from these data.

Appendix

THE LUNGS AND THEIR EXPECTORATIONS

Besides *proteins* and the *albuminoids* of the connective-substance group, *lecithin*, *taurine* (especially in ox-lungs), *uric acid*, and *inosite* have been found in the lungs. POULET[1] claims to have found a special acid in the lung-tissue, which he has called *pulmotartaric acid*. Glycogen occurs abundantly in the embryonic lung, but is absent in the adult organ. The proteolytic enzymes also belong to the physiological constituents of the lungs. They are active in the autolysis of the lungs (JACOBY) as well as in the solution of pneumonic infiltrations (FR. MÜLLER).[2]

The lungs have a strong reducing property, which BOHR explains by the extensive oxidation processes in the lungs. According to N. SIEBER they also have the ability to decompose neutral fats, while RIEHL[3] says they do not have the ability to invert milk sugar.

The black or dark-brown pigment in the lungs of human beings and domestic animals consists chiefly of carbon, which originates from the soot in the air. The pigment may in part also consist of melanin. Besides carbon, other bodies, such as iron oxide, silicic acid, and clay, may be deposited in the lungs, being inhaled as dust.

Among the bodies found in the lungs under pathological conditions must be specially mentioned, proteoses (and peptones?) in pneumonia and suppuration, glycogen, a slightly dextrorotatory carbohydrate differing from glycogen, found by POUCHET in consumptives, and finally also cellulose, which, according to FREUND,[4] occurs in the lungs, blood, and pus of persons with tuberculosis.

C. W. SCHMIDT found in 1000 grams of mineral bodies from the normal human lung the following: NaCl 130, K_2O 13, Na_2O 195, CaO 19, MgO 19, Fe_2O_3 32, P_2O_5 485, SO_3 8, and sand 134 grams. According to OIDTMANN,[5] the lungs of a 14-day old child contained 796.05 p. m. water, 198.19 p. m. organic bodies, and 5.76 p. m. inorganic bodies.

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[1] Cited from Maly's Jahresber., **18**, 248.

[2] Jacoby, Zeitschr. f. physiol. Chem., **33**; Müller, Verhandl. d. Kongress. f. inn. Medizin, 1902.

[3] N. Sieber, Zeitschr. f. physiol. Chem., **55**; Riehl, Zeitschr. f. Biol., **48**.

[4] Pouchet, Compt. Rend., **96**; Freund, cited from Maly's Jahresber., **16**, 471.

[5] Schmidt, cited from v. Gorup-Besanez, Lehrbuch, 4. Aufl., 727; Oidtmann, *ibid.*, 732.

The sputum is a mixture of the mucous secretion of the respiratory passages, of saliva and buccal mucus. Because of this its composition is variable, especially under pathological conditions when various products mix with it. The chemical constituents are, besides the mineral substances, chiefly mucin with a little proteid and nuclein substance. Under pathological conditions proteoses and peptones (?), which are probably produced by bacterial action or by autolysis (WANNER, SIMON[1]), volatile fatty acids, glycogen, CHARCOT'S crystals, and also crystals of cholesterin, hæmatoidin, tyrosine, fat and fatty acids, triple phosphates, etc., have been found.

The form constituents are, under physiological circumstances, epithelium-cells of various kinds, leucocytes, sometimes also red blood-corpuscles and various kinds of fungi. In pathological conditions elastic fibers, spiral formations consisting of a mucin-like substance, fibrin coagulum, pus, pathogenic microbes of various kinds and the above-mentioned crystals occur.

The lung concretions contain chiefly calcium and phosphoric acid as inorganic constituents. Silicic acid is, in ZICKGRAF'S opinion, an essential and constant constituent, but according to GERHARTZ and STRIGEL [2] is not always constant.

III. HOW ARE THE PHYSIOLOGICAL OXIDATION PROCESSES BROUGHT ABOUT?

After the oxygen passes from the blood to the tissues a very extensive oxidation is there carried out, which in conjunction with cleavage processes yields finally the products carbon dioxide, water, urea and other bodies. Little is known as to the manner in which the organism carries out such complete oxidations. Attempts have been made for a long time to explain the mechanism of the oxidation processes. Thus SCHÖNBEIN [3] believed in the presence in the organism of oxygen in a peculiar form, suited for the oxidation. HOPPE-SEYLER [4] connects the oxidation with a simultaneous reduction; reducible or readily oxidizable substances first rupture the oxygen molecule ($=O_2$) into atoms and take one up; the other at the moment it is set free is especially able to oxidize. M. TRAUBE [5] believes that in the case that a readily oxidizable (auto-oxidizable) substance is present, the oxidation is produced by means of the entire oxygen molecule, and indeed in the manner that water is transformed at the same time to hydrogen peroxide, for example $A+2H_2O+O_2=A(OH)_2+H_2O_2$.

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[1] Wanner, Deutsch. Arch. f. klin. Med., **75**; Simon, Arch. f. exp. Path. u. Pharm., **49**.

[2] Gerhartz and Strigel, Beitr. z. klin. d. Tuberkulose, **10**, which also cites Zickgraf.

[3] Baseler, Verh., Bd. 1, 339 (1853); Sitzungsber. Bayer. Akad. Wiss., 1863, Bd. 1, 274.

[4] Zeitschr f. physiol. Chem., **2**, 1 (1878).

[5] Ber. d. d. chem. Gesellsch., Bd. **15** to **26** (1882 to 1893).

With these views as basis and at the same time although independently of each other ENGLER[1] and BACH[2] have developed a theory which for the present is the one generally accepted. According to this theory, peroxides of the hydrogen peroxide type are always formed as primary oxidation products. The peroxides are either formed by a direct attachment of oxygen with readily oxidizable substances or in consequence of a simultaneous oxidation with other substances—in the last way, for example, the formation of H_2O_2 in the oxidation of indigo-white to indigo according to the formula:

$$\underset{\text{Indigo-white}}{\text{Indigo}\begin{matrix}H\\H\end{matrix}} + O_2 = \text{Indigo} + H_2O_2$$

Only certain substances have the ability either directly or indirectly of forming peroxides. Certain protein-like substances occurring especially in the plants which have this ability, have been called *oxygenases* by BACH and CHODAT.[3] Most of the substances which are oxidized within the organism lose their ability to be directly oxidized. The oxidation of such substances can, according to BACH,[4] be accomplished in that the oxygen is transported to the substance to be oxidized from the peroxide simultaneously present by means of special enzymes, the *peroxidases*. These latter were first prepared from pumpkins and from horse-radish roots. In the absence of peroxides or oxygenases the peroxidases are without action. CHODAT and BACH[5] have also found that certain preparations, which have previously been called *oxidases*, can be decomposed into oxygenases and peroxidases. According to BACH'S theory the formation of peroxides is a constant process going on in the organism, to which the organism accommodates itself, in that the cells by means of the peroxidases can make use of the peroxides for the oxidation processes. Besides this the organism also forms other enzymes, the so-called *catalases*, which have the ability of decomposing the peroxides with the formation of molecular oxygen (O_2) and in this way making a possible excess of peroxides harmless.[6] In reference to the behavior of the perox-

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[1] Verh. naturw. Verein, Karlsruhe, **20**, XI (1896), Bd. **12**, 72; see also Zeitschr f. physiol. Chem., **59**, 327 (1909).

[2] Compt. Rend., **124**, 951 (1897); see also Bioch. Centralbl., **1**, 417, and 457 (1903); **9**, 1 (1909).

[3] Ber. d. d. chem. Gesellsch., **36**, 600 (1903).

[4] *Ibid.*, **36**, 600 (1903).

[5] *Ibid.*, **36**, 606 (1903).

[6] Bioch. Centralbl., **1**, 460.

idases to heat the views are contradictory. CZYHLARZ and v. FÜRTH found that the peroxidases from animal tissues were remarkably resistant to high temperatures, while BATELLI and STERN [1] find that animal peroxidases are destroyed even at 66° C.

According to BACH'S theory on the one hand, substances are necessary for the oxidation, which take up oxygen with the formation of peroxides (oxygenases) and on the other hand, substances which are able to transport the oxygen from the peroxides to the oxidizable bodies (peroxidases). In certain oxidations, for example in the phenols, the peroxidases can be replaced by certain metallic combinations.[2] The iron, of the blood pigments, acts in this way in the guaiacum reaction (see Chapter XIV). The oil of turpentine here represents the peroxide and can be replaced by hydrogen peroxide. The oxidizable substance, which becomes blue in the reaction, is the guaiaconic acid in the guaiac gum.[3]

Irrespective of whether the division of the oxidation enzymes into oxygenases and peroxidases can be carried out in all cases, there are various oxidation processes, whose occurrence by a combination of oxygenase (or peroxide) with peroxidase (or metallic salt) can be explained only with difficulty. According to BERTRAND'S [4] view the action of plant oxidation enzymes is connected with their manganese content. Nevertheless BACH [5] has been able to prepare enzymes from plants which were entirely free from iron as well as manganese salts. Starting from BERTRAND'S view, TRILLAT [6] has prepared solutions of manganese salts, alkali and colloidal substances, which acted like oxidizing enzymes. DONY-HÉNAULT [7] has prepared artificial "oxidases" from a faintly alkaline solution of gum treated with a solution of manganese salt. According to EULER and BOLIN [8] the salts of certain organic acids have the ability of setting the oxidation power of manganese salts free. Similar observations have been made by WOLFF.[9] In the oxidation of auto-oxidizable substances the presence of extremely small amounts of iron salts may be of advantage,

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[1] Czyhlarz and v. Fürth, Hofmeister's Beiträge, **10**, 358 (1907); Batelli and Stern, Bioch. Zeitschr., **12**, 44 (1908).

[2] Ber. d. d. chem. Gesellsch., **43**, 366 (1910).

[3] C. E. Carlson, Zeitschr. f. physiol. Chem., **48**, 69 (1906). P. Richter, Arch. d. Pharm., **244**, 90 (1906).

[4] Compt. Rend., **124**, 1032, 1355 (1897).

[5] Ber. d. d. chem. Gesellsch., **43**, 364 (1910).

[6] Compt. Rend., **138**, 274 (1904).

[7] Bull. acad. roy. de Belgique, 1908, 105.

[8] Zeitschr. f. physiol. Chem., **57**, 80 (1908).

[9] Ann. inst. Past., **24** (1910).

for example with the lecithins (THUNBERG, WARBERG and MEYERHOF[1] as well as in the oxidation of certain thio-compounds.[2]

BATELLI and STERN[3] have made careful investigations as to the occurrence of peroxidases in the animal organism. In order to eliminate the action of catalases which are present in the tissues and which, as shown by earlier investigators, decompose the hydrogen peroxide, these experimenters used ethyl hydrogen peroxide, $C_2H_5.O.O.H$, on which the catalases do not act. With ethyl hydrogen peroxide and hydroidic acid nearly all animal tissues gave the peroxidase reaction, wherein free iodine was formed. SCHEUNERT, GRIMMER and ANDRYEWSKI[4] make use of the following solution as a reagent for peroxidases: 100 cc. fresh tincture of guaiacum and 0.1 to 0.2 cc. 3 per cent H_2O_2 solution. Blood does not give any blue coloration with this reagent, but in the presence of large quantities of H_2O_2 or other superoxide solutions (ethylhydrogen peroxide, oil of turpentine) it does give a blue coloration. With this active tincture of guaiacum these experimenters were able to detect peroxidases in the salivary glands, as well as the mucous membrane of the stomach and intestine of certain varieties of animals. The liver was always free from peroxidases. On the other hand, BATELLI and STERN[5] also tested the ability of various tissues of acting upon formic acid in the presence of H_2O_2 with the evolution of carbon dioxide. In later works these experimenters claim that in all animal tissues there exists a substance of an unknown nature, the *pnein*, which has the ability of bringing about the respiration in all animal tissues. Pnein, which is soluble in water, dializable and resistant to temperature, increases the so-called *chief respiration*, which is connected with the life of the cells and which stops more or less rapidly after the death of the animal. The so-called *accessory respiration* continues quite a long time after death, and this can continue in the absence of cell elements and is of an enzymotic character. THUNBERG[6], who has constructed an apparatus for measuring the respiratory exchange of gas in small organs and organisms (*microrespirometer*) finds that the salts of certain organic acids (succinic acid, citric acid, malic acid, fumaric acid) accelerate more or less the gas exchange in surviving frog's muscles. In their last communication BATELLI and STERN[7] differentiate between two kinds of oxidation catalysts: the *oxidases* and the *oxidones*. The first to which, among others the tyrosinase, alcohol-

[1] Skand. Arch. f. Physiol., **24**, 90 (1911); Zeitschr. f. physiol. Chem., **85**, 412 (1913).
[2] Thunberg, Lunds Univ. Årsskr., N. F., 2, Bd. **9** (1913).
[3] Bioch. Zeitschr., **13**, 44 (1908).
[4] *Ibid.*, **53**, 300 (1913).
[5] *Ibid.*, **21**, 487 (1910); **30**, 172 (1910); **33**, 315 (1911).
[6] Skand. Arch. f. Physiol., **17**, **23**, **24**, **25** (1911).
[7] Bioch. Zeitschr., **46**, 317, 343 (1912); Compt. rend., soc. biol., **74**, 212 (1913).

oxidase, xanthin oxidase (see below) belong, are soluble in water, resistant to alcohol and acetone and can be heated to 55–60° C. The oxodones, which for example oxidize succinic acid to malic acid and act upon *p*-phenyldiamine, cannot be extracted from the tissues by water; they are injured or destroyed by alcohol, acetone or by being heated to 55–60° C.

WARBURG [1] has carried on extensive experiments on the influence of foreign bodies upon the respiration in the cells and has conformed the results to OVERTON's theory of lipoid membrane.

No deep oxidation processes have been produced under the influence of the mentioned oxidizing substances outside of the organism. The various divergent views on the nature of the oxidizing substances strikingly indicates how little exact knowledge we have of this subject. Perhaps the oxidation within the body takes place step by step, and it seems possible that the consecutive stages of the reaction can be brought about by different agents. A positive division of the so-called oxidizing enzymes cannot be made at the present time. For in many cases it is undecided whether we are dealing with enzymotic processes or with non-enzymotic catalytic action of metallic salts, especially as the reports on the heat-resistance of the active substances are contradictory. In the enzymotic oxidations we are in many cases in doubt whether the oxygen is transported directly to the oxidizing substance or whether the oxidation is brought about by the system peroxide plus peroxidase. When the oxidation cannot be shown as due to the just-mentioned system, then the active enzyme is called simply *oxidase*.

In consideration of the substances upon which the oxidation enzymes act, we can divide them for the present into the following groups, according to OPPENHEIMER:[2]

1. *Alcoholases*, which transform alcohols into acids, for example the acetic-acid-forming enzyme of certain varieties of bacteria.
2. *Aldehydases*, which oxidize aldehydes into acids, for example, salicylase.
3. *Purine-oxidases*, which oxidize hypoxanthine and xanthine into uric acid and which act upon uric acid with the formation of allantoin. This last reaction is produced by the action of the so-called *uricase*.
4. *Phenol-oxidases*, which oxidize various phenols and related bodies with the formation of pigments. The guaiac reaction is of this kind.
5. *Tyrosinases*, which oxidize tyrosine and closely related bodies into dark pigments.

The system peroxide+peroxidase has been shown only in connection with enzymes of group 4.

Besides the above-mentioned bodies, upon which the different classes of oxidizing enzymes act more specifically, we can mention the following as oxidase reagents.

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[1] Zeitschr. f. physiol. Chem., 67, 69, 70, (1910); 71, 76 (1911).

[2] Die Fermente und ihre Wirkungen, 3. Aufl., Spec. Teil, 351 (1909).

1. Iodides in acid solution in the presence of H_2O_2. According to BACH and CHODAT [1] this reaction is completely parallel with the guaiac reaction.

2. Formic acid with H_2O_2 (see above).

3. Amines (especially *p*-phenyldiamine) which forms colored products on taking up oxygen.[2]

4. Leucobases or mixtures of their formers, which by oxidation are converted into pigments. A solution of a mixture of α-naphthol and *p*-phenylen-diamine made alkaline with soda gives indophenol on taking up oxygen (RÖHMANN and SPITZER [3]).

5. Certain benzene derivatives which on oxidation and loss of water are transformed into diphenol derivatives, for example vanillin into dehydrodivanillin.[4]

For quantitative estimation of the extent of oxidation BACH and CHODAT [5] use the transformation of pyrogallol into purpurogallin, which latter can be weighed. BACH [6] determines the amount of iodine set free in the reaction between hydrogen peroxide and hydriodic acid and BATELLI and STERN [7] determine the quantity of CO_2 formed in the oxidation of formic acid.

There is no doubt that reductions occur to a great extent in the animal body and often go hand in hand with oxidations. The question as to the extent in which special reduction enzymes are concerned, is still undecided. As the oxidations are explained by the action of special enzymes, so also we can admit of special reduction enzymes, so-called *reductases* or *hydrogenases*. To this group belongs the so-called "philothion" (De REY-PAILHADE), which in the presence of sulphur and water develops sulphuretted hydrogen, while others on the contrary do not accept this view, and deny the enzyme nature of philothion.[8] The investigations of NASSE and RÖSING [9] on the oxidation of protein in the presence of sulphur contradict the enzymotic nature of this formation of sulphuretted hydrogen, and the recent investigations of HEFFTER [10] have shown that certain reductions occurring in the tissues are not produced by enzymes. He has also shown that those reductions, which are not influenced by HCN, like the reduction of pigments (methylene blue),

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[1] Ber. d. d. chem. Gesellsch., **35**, 2466 (1902).

[2] Bioch. Zeitschr., **46**, 317 (1912).

[3] Ber. d. d. chem. Gesellsch., **28**, 567 (1894).

[4] In regard to this and other reagents, see Zeitschr. f. physiol. Chem., **59**, 359 (Engler and Herzog).

[5] Ber. d. d. chem. Gesellsch., **37**, 1342 (1904).

[6] *Ibid.*, **37**, 3785 (1904).

[7] Bioch. Zeitschr., **31**, 443; **33**, 282 (1911).

[8] De Rey-Pailhade, Recherches expér. sur le Philothion, etc., Paris (G. Masson), 1891, and Nouvelles recherches sur le Philothion, Paris (Masson), 1892; Bull. soc. chim. (4), 1; Pozzi-Escot, Bull. soc. chim. (3), **27**, and Chem. Centralbl., **1904**, **1**, 1645; Chodat and Bach, Ber. d. d. chem. Gesellsch., **36**; Abelous and Ribaut, Compt. Rend., **137**, and Bull. soc. chim., (3), **31**.

[9] E. Rösing, Unters. über die Oxydation von Eiweiss in Gegenwart von Schwefel, Inorg.-Dissert, Rostock, 1891.

[10] Med.-naturw. Arch., **1**, 81–104; Marburg, cited in Chem. Centralbl., **2**, 1907, 822; Thunberg, Ergebn. d. Physiol., **11**.

of sulphur to H_2S and others, can be brought about by the labile H of the sulphydryl-compounds. In this manner for example the cysteine (see Chapter II) acts and quickly reacts with sulphur with the formation of H_2S and similarly acting substances have been detected by HEFFTER in various organs and organ extracts. We have here a group of reductions which are not of an enzyme nature.

From the investigations of ABELOUS and ALOY[1] it follows that plants as well as animal organs have the ability at the same time of oxidizing salicylaldehyde and of reducing nitrates to nitrites. On the other hand SCHARDINGER, TROMMSDORFF and BACH[2] have shown that fresh cow's milk, which alone is without action upon methylene blue and on nitrates, reduces these bodies in the presence of aldehydes into leucobases or nitrites. Boiled milk does not have this power and the action is explained by the presence of a *reductase*, the so-called SCHARDINGER enzyme. The optimum of action lies at about 70° C. BACH found the same action in various animal organs. The process may to be just as well considered as an oxidation under the influence of an aldehydase whereby the methylene blue or the nitrate gives up the oxygen for the oxidation of the aldehyde. On the other hand STRASSNER[3] ascribes the reduction of the methylene blue to the above-mentioned reducing action of the sulphydryl groups.

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[1] Compt. Rend., **138**, 382 (1904); see also Pozzi-Escot, *ibid.*, **138**, 511.

[2] Schardinger, Zeitschr. f. Unters. d. Nahrungs- und Genussmittel, **5**, 22 (1902); Trommsdorff, Centralbl. f. Bakter., **49**, 291 (1909); Bach, Ber. d. d. chem. Gesellsch., **42**, 4463 (1909); Bioch. Zeitschr., **31**, 443; **33**, 282; **38**, 154 (1911).

[3] *Ibid.*, **29**, 295 (1910).

CHAPTER XVII.

METABOLISM WITH VARIOUS FOODS, AND THEIR NECESSITY TO MAN.

I. GENERAL DISCUSSION AND METHODS USED IN THE STUDY OF MATTER AND FORCE METABOLISM.

THE conversion of chemical energy into heat and mechanical work which characterizes animal life, leads to the formation of relatively simple compounds—carbon dioxide, urea, etc.—which leave the organism, and which, moreover, being very poor in energy, are for this reason of little or no value to the body. It is therefore absolutely necessary for the continuance of life and the normal course of the functions of the body that the organism and its different tissues should be supplied with new material to replace that which has been exhausted. This is accomplished by means of food. Those bodies are designated as *food* which have no injurious action upon the organism and which serve as a source of energy and can replace those constituents of the body that have been consumed in metabolism or that can prevent or diminish the consumption of such constituents.

Among the numerous dissimilar substances which man and animals take with the food all cannot be equally necessary or have the same value. Some perhaps are unnecessary, while others may be indispensable. We have learned by direct observation and a wide experience that besides the oxygen, which is necessary for oxidation, the essential foods for animals in general, and for man especially, are *water*, *mineral bodies*, *proteins*, *carbohydrates*, and *fats*.

It is also apparent that the various groups of food-stuffs necessary for the tissues and organs must be of varying importance; thus, for instance, water and the mineral bodies have another value than the organic foods, and these again must differ in importance among themselves. The knowledge of the action of various nutritive bodies on the exchange of material from a qualitative as well as a quantitative point of view must be of fundamental importance in determining the value of different nutritive substances relative to the demands of the body for food under various conditions, and also in deciding many other questions—for instance, the proper nutrition for an individual in health and in disease.

Such knowledge can be attained only by a series of systematic and thorough observations, in which the quantity of nutritive material, relative to the weight of the body, taken and absorbed in a given time is compared with the quantity of final metabolic products which leave the organism at the same time. Researches of this kind have been made by investigators, but above all should be mentioned those made by BISCHOFF and VOIT, by PETTENKOFER and VOIT, and by VOIT and his pupils, by RUBNER, ZUNTZ and by ATWATER.

It is absolutely necessary in researches on the exchange of material to be able to collect, analyze, and quantitatively estimate the excreta of the organism, so that they may be compared with the quantity and composition of the nutritive bodies ingested. In the first place, one must know what the habitual excreta of the body are and in what way these bodies leave the organism. One must also have trustworthy methods for their quantitative estimation.

The organism may, under physiological conditions, be exposed to accidental or periodic losses of valuable material—such losses as occur only in certain individuals, or in the same individual only at a certain period; for instance, the secretion of milk, the production of eggs, the ejection of semen or menstrual blood. It is therefore apparent that these losses can be the subject of investigation and estimation only in special cases.

The regular and constant excreta of the organism are of the very greatest importance in the study of metabolism. To these belong, in the first place, the true final metabolic products—*carbon dioxide*, *urea* (uric acid, hippuric acid, creatinine, and other urinary constituents), and a part of the *water*. The remainder of the water, the *mineral bodies*, and those secretions or tissue constituents—*mucus*, *digestive fluids*, *sebum*, *perspiration*, and *epidermal formations*—which are either poured into the intestinal tract, or secreted from the surface of the body, or broken off and thereby lost to the body, also belong to the constant excreta.

The remains of food, sometimes indigestible, sometimes digestible but not acted upon, which are contained in the feces, and which vary considerably in quantity and composition with the nature of the food, also belong to the excreta of the organism. Even though these remains, which are never absorbed and therefore are never constitutents of the animal fluids or tissues, cannot be considered as excreta of the body in a strict sense, still their quantitative estimation is absolutely necessary in certain experiments on the exchange of material.

The determination of the constant loss is in some cases accompanied with the greatest difficulties. The loss from the detached epidermis, from the secretion of the sebaceous glands, etc., cannot be determined with exactness without difficulty, and therefore—as they do not occasion any appreciable loss because of their small quantity—they need not be considered in quantitative experiments on metabolism. This also applies to the constituents of the mucus, bile, pancreatic and intestinal juices, etc., occurring in the contents of the intestine, and which, leaving the body with the feces, cannot be separated from the other contents of

the intestine and therefore cannot be quantitatively determined separately. The uncertainty which, because of the intimated difficulties, attaches itself to the results of the experiment, is very small as compared to the variation which is caused by different individualities, different modes of living, different foods, etc. Only approximate values can therefore be given for the constant excreta of the human body.

The following figures represent the quantity of excreta for twenty-four hours from a grown man, weighing 60–70 kilos, on a mixed diet. The figures are compiled from the results of different investigators:

	Grams.
Water	2500–3500
Salts (with the urine)	20–30
Carbon dioxide	750–900
Urea	20–40
Other nitrogenous urinary constituents	2–5
Solids in the excrement	20–50

These total excreta are approximately divided among the various excretions in the following way; but still it must not be forgotten that this division may vary to a great extent under different external circumstances: By *respiration* about 32 per cent, by the *evaporation from the skin* 17 per cent, with the *urine* 46–47 per cent, and with the *excrement* 5–9 per cent. The elimination by the skin and lungs, which is sometimes differentiated by the name "*perspiratio insensiblis*" from the visible elimination by the kidneys and intestine, is on an average about 50 per cent of the total elimination. This proportion, quoted only relatively, is subject to considerable variation, because of the great difference in the loss of water through the skin and kidneys under varying circumstances.

The nitrogenous constituents of the excretions consist chiefly of urea, or uric acid in certain animals, and the other nitrogenous urinary constituents. A disproportionately large part of the nitrogen leaves the body with the urine, and, as the nitrogenous constituents of this excretion are final products of the metabolism of proteins in the organism, the quantity of proteins catabolized in the body may be easily calculated by multiplying the quantity of nitrogen in the urine by the coefficient 6.25 ($\frac{100}{16}=6.25$), if it is admitted that the proteins contain in round numbers 16 per cent of nitrogen.

Still another question is whether the nitrogen leaves the body only with the urine or by other channels. The latter is habitually the case. The discharges from the intestine always contain some nitrogen, which consists in part of non-absorbed remnants of the food, but in chief part and sometimes entirely of constituents of the epithelium and the secretions. Under these circumstances it is apparent that one cannot give any exact figures which are valid for all cases for that part of the nitrogen of the excrement which originates in the digestive tract and in the digestive

fluids. It may not vary in different individuals only, but also in the same individual after more or less active secretion and absorption. In the attempts made to determine this part of the nitrogen of the excrement it has been found that in man, on non-nitrogenous or nearly nitrogen-free food, it amounts in round numbers to somewhat less than 1 gram per twenty-four hours (RIEDER, RUBNER). Even with such food the absolute quantity of nitrogen eliminated by the feces increases with the quantity of food because of the accelerated digestion (TSUBOI [1]), and is greater than in starvation. MÜLLER [2] found in his observations on the faster CETTI that only 0.2 gram nitrogen was derived from the intestinal canal.

The quantity of nitrogen which leaves the body under normal circumstances by means of the hair and nails, with the scaling off of the skin, and with the perspiration cannot be accurately determined. It is nevertheless so small that it may be ignored. Only in profuse sweating need the elimination by this channel be taken into consideration.

The view was formerly held that in man and carnivora an elimination of gaseous nitrogen took place through the skin and lungs, and because of this, on comparing the nitrogen of the food with that of the urine and feces, a *nitrogen deficit* occurred in the visible elimination.

This question has been the subject of much discussion and of numerous investigations, the most recent by KROGH and OPPENHEIMER.[3] These researches have shown that the above assumption is unfounded, and moreover several authorities, especially PETTENKOFER and VOIT, and GRÜBER,[4] have shown by experiments on man and animals that with the proper quantity and quality of food the body can be brought into *nitrogenous equilibrium*, in which the quantity of nitrogen voided with the urine and feces is equal or nearly equal to the quantity contained in the food. Undoubtedly we must admit, with VOIT, that a deficit of nitrogen does not exist, or it is so insignificant that in experiments upon metabolism it need not be considered. Ordinarily, in investigations on the catabolism of proteins in the body, it is only necessary to consider the nitrogen of the urine and feces, but it must be remarked that the nitrogen of the urine is a measure of the extent of the catabolism of the proteins

[1] Rieder, Zeitschr. f. Biologie, **20**; Rubner, *ibid.*, **15**; Tsuboi, *ibid.*, **35**.

[2] Berlin. klin. Wochenschr., 1887.

[3] See Regnault and Reiset, Annal. d. chem. et phys. (3), **26**, and Annal. d. Chem. u. Pharm., **73**; Seegen and Nowak, Wien. Sitzungsber., **71**, and Pflüger's Arch., **25**; Pettenkofer and Voit, Zeitschr. f. Biologie, **16**; Leo, Pflüger's Arch., **26**; Krogh, Skand. Arch. f. Physiol., **18**, and Wien. Sitz. Ber., **115**, III; Oppenheimer, Bioch. Zeitschr., **4**.

[4] Pettenkofer and Voit, in Herrman's Handbuch, **6**, Thl. 1; Grüber, Zeitschr. f. Biologie, **16** and **19**.

in the body, while the nitrogen of the feces (after deducting about 1 gram on a mixed diet) is a measure of the non-absorbed part of the nitrogen of the food. The nitrogen of the food, as well as of the excreta, is generally determined by KJELDAHL'S method.

In the oxidation of the proteins in the organism, their sulphur is oxidized into sulphuric acid, and on this depends the fact that the elimination of sulphuric acid by the urine, which in man is but to a small extent derived from the sulphates of the food, nearly makes equal variations with the elimination of nitrogen by the urine. If the amount of nitrogen and sulphur in the proteins is considered as 16 per cent and 1 per cent respectively, then the proportion between the nitrogen of the proteins and the sulphuric acid, H_2SO_4, produced by their combustion is in the ratio 5.2 : 1, or about the same as in the urine (see page 765). The determination of the quantity of sulphuric acid eliminated in the urine gives us an important means of controlling the extent of the transformation of proteins, and such a control is especially important in cases in which it is expected to study the action of certain nitrogenous non-albuminous bodies on the metabolism of proteins, or to decide the question whether a true protein combustion and not only a washing out of the nitrogenous products of metabolism from the tissues is taking place. A determination of the nitrogen alone is naturally not sufficient in such cases. A perfectly positive measure of the protein catabolism cannot be made from the sulphuric acid of the urine, as the various protein substances have a rather variable sulphur content, and on the other hand also a variable quantity of the sulphur in the urine exists as so-called neutral sulphur.

In metabolism experiments the total sulphur of the urine as well as the feces must be determined, and it may also be of importance to determine the relation between the sulphuric-acid sulphur and the neutral sulphur of the urine. The elimination of the sulphur originating from the proteins does not, according to v. WENDT, HÄMÄLÄINEN and HELME and CH. WOLFF[1] always run parallel with the protein nitrogen, and for the white of egg the maxima of the elimination curves may indeed be separated during a period of twenty-four hours (WOLFF). The sulphur is more quickly eliminated than the nitrogen, and this behavior of sulphur gives in certain cases a more positive picture of the temporal catabolism of protein than the nitrogen. This is of importance, as the elimination of the nitrogen corresponding to a certain amount of protein requires several days for completion. FALTA has also observed that the chief amount of nitrogen in man on taking different proteins is secreted with varying rapidity, and the same is true, according to HÄMÄLÄINEN

[1] Wendt, Skand. Arch. f. Physiol., **17**; Hämäläinen and Helme, *ibid.*, **19**; Falta, Deutsch. Arch. f. klin. Med., **86**; Ch. G. Wolff, Bioch. Zeitschr., **40**.

and HELME, for the elimination of sulphur, as in their experiments the sulphur elimination from white of egg required about six days and from casein only two days. These conditions must be considered in metabolism experiments.

Besides lecithins and other phosphatides the body takes with its food pseudonucleins as well as true nucleins, and these are absorbed more or less completely from the intestinal tract and then assimilated. On the other hand, the phosphorized protein substances, lecithins and phosphatides, are also decomposed within the body, and their phosphorus is chiefly eliminated as phosphoric acid and also in part as organic phosphorus (see page 757). For these reasons the phosphorus is of great importance in certain investigations on metabolism.

It is found, on comparing the nitrogen of the food with that of the urine and feces, that there is an excess of the first; this means that the body has increased its stock of nitrogenous substances—proteins. If, on the contrary, the urine and feces contain more nitrogen than the food taken at the same time, this denotes that the body is giving up part of its nitrogen—that is, part of its own proteins has been decomposed.

We can, from the quantity of nitrogen, as above stated, calculate the corresponding quantity of proteins by multiplying by 6.25.[1] Usually, according to VOIT'S proposition, the nitrogen of the urine is not calculated as decomposed proteins, but as decomposed muscle-substance or flesh. Lean meat contains on an average about 3.4 per cent nitrogen; hence each gram of nitrogen of the urine corresponds in round numbers to about 30 grams of flesh. The assumption that lean meat contains 3.4 per cent nitrogen is arbitrary, and the relation of N : C in the proteins of dried meat, which is of great importance in certain experiments on metabolism, is given differently by various experimenters, namely, 1 : 3.22–1 : 3.68. ARGUTINSKY found in beef, after complete removal of fat and subtraction of glycogen, that the relation was 1 : 3.24 (see Chapter X).

The carbon leaves the body chiefly as carbon dioxide, which is eliminated by the lungs and skin. The remainder of the carbon is excreted in the urine and feces in the form of organic compounds, in which the quantity of carbon can be determined by elementary analysis. It was formerly considered sufficient to calculate the quantity of carbon in the urine from the quantity of nitrogen according to the relation $N:C=1:0.67$ to 0.72. This does not seem to be trustworthy, as this relation varies and depends, according to TANGL, PFLÜGER, LANGSTEIN, and STEINITZ,[2] upon the kind of food. TANGL has shown that the richer the food is in carbohydrates the more carbon and hence the more heat of combustion per

[1] In calculating the protein catabolism from the nitrogen of the urine it must not be forgotten that the food often contains nitrogenous extractives whose nitrogen cannot be calculated as protein and for which a special correction must be made, if necessary.

[2] Tangl, Arch. f. (Anat. u.) Physiol., 1899, Suppl. Bd.; Pflüger in Pflüger's Arch., 79; Langstein and Steinitz, Centralbl. f. Physiol., 19.

gram of nitrogen does the urine contain. He found the following for 1 gram of nitrogen in the urine: With diet rich in fat 0.747 gram C and 9.22 calories; for carbohydrate-rich diet he found 0.936 gram C and 11.67 calories. The quantity of carbon in the feces can be calculated from the quantity of nitrogen in the feces by using the quotient $\frac{C}{N}=9.2$ (average with mixed diet, according to ATWATER and BENEDICT.[1])

The extent of the gas exchange can be determined by any of the methods given on page 869. By multiplying the quantity of carbon dioxide found by 0.273 one obtains the quantity of carbon eliminated as CO_2. If the total quantity of carbon eliminated in various ways is compared with the carbon contained in the food, some idea can be obtained as to the transformation of the carbon compounds. If the quantity of carbon in the food is greater than in the excreta, then the excess is deposited; while if the reverse be the case, it shows a corresponding loss of body substance.

The nature of the substances here deposited or lost, whether they consist of proteins, fats, or carbohydrates, is learned from the total quantity of the nitrogen of the excretions. The corresponding quantity of proteins may be calculated from the quantity of nitrogen, and, as the average quantity of carbon in the proteins is known, the quantity of carbon which corresponds to the decomposed proteins may be easily ascertained. If the quantity of carbon thus found is smaller than the quantity of the total carbon in the excreta, it is then obvious that some other nitrogen-free substance has been consumed besides the proteins. If the quantity of carbon in the proteins is considered in round numbers as 52.5 per cent, then the relation between carbon (52.5) and nitrogen (16) is 3.28, or in round numbers 3.3 : 1. If the total quantity of nitrogen eliminated is multiplied by 3.3, the excess of carbon in the eliminations over the product found represents the carbon of the decomposed non-nitrogenous compounds. For instance, in the case of a person experimented upon, 10 grams of nitrogen and 200 grams of carbon were eliminated in the course of twenty-four hours; then these 62.5 grams of protein correspond to 33 grams of carbon, and the difference, $200-(3.3\times10)=167$, represents the quantity of carbon in the decomposed non-nitrogenous compounds. If we start from the simplest case, starvation, where the body lives at the expense of its own substance, then, since the quantity of carbohydrates as compared with the fats of the body is extremely small, in such cases in order to avoid mistakes the assumption must be made that the person experimented upon has used only fat and proteins. As animal fat contains on an average 76.5 per cent carbon, the quantity of transformed fat may be calculated by multiplying the carbon by $\frac{100}{76.5}=1.3$. In the case of the above example, the person experimented upon would have used 62.5 grams of proteins and $167\times1.3=217$ grams of fat, of his own body, in the course of the twenty-four hours.

Starting from the nitrogen balance, it can be calculated in the same way whether an excess of carbon in the food as compared with the quantity of carbon in the excreta is retained by the body as proteins or fat or as both. On the other hand, with an excess of carbon in the excreta one can determine how much of the loss of the substance of the body is due to a consumption of the proteins on the one side and of non-nitrogenous bodies on the other side. How to especially

[1] Bull. of Dept. of Agric., U. S., Washington, No. 136.

calculate the part taken by the fats and carbohydrate will be shown in connection with the calculation of the energy metabolism.

The quantity of water and mineral bodies voided with the urine and feces can easily be determined. The quantity of water eliminated by the skin and lungs may be directly estimated by means of the large respiration apparatus.

The organic constituents of the body as well as the foodstuffs introduced, represent a sum of chemical energy which the body can use for force. The exchange of material is also an exchange of force, and the first stands in such close relation to the second that the study of one cannot be separated from the other. The energy theory of metabolism has exercised an extraordinarily fruitful influence upon the entire study of metabolism and nutrition, and this is due in great measure to the work of RUBNER.

This energy of the various foods may be represented by the amount of heat which is set free in their combustion. This quantity of heat is expressed as calories, and a small calorie is the quantity of heat necessary to warm 1 gram of water from 0° to 1° C. A large calorie is the quantity of heat necessary to warm 1 kilo of water 1° C. Here and in the following pages large calories are to be understood. There are numerous investigations by different experimenters, such as FRANKLAND, DANILEWSKI, RUBNER, BERTHELOT, STOHMANN, BENEDICT and OSBORNE, and others, on the calorific value of different foodstuffs. The following results, which represent the calorific value of a few nutritive bodies on complete combustion outside of the body to the highest oxidation products, are taken from STOHMANN'S [1] work.

	Calories.
Casein	5.86
Ovalbumin	5.74
Conglutin	5.48
Protein (average)	5.71
Animal tissue-fat	9.50
Butter-fat	9.23
Cane-sugar	3.96
Milk-sugar	3.95
Glucose	3.74
Starch	4.19

Fats and carbohydrates are completely burnt in the body, and one can therefore consider their combustion equivalent as a measure of the living force developed by them within the organism. We generally designate 9.3 and 4.1 calories for each gram of substance as the average for the physiological calorific value of fats and carbohydrates respectively.

[1] See Rubner, Zeitschr. f. Biologie, **21**, which also cites the works of Frankland and Danilewski; see also Berthelot, Compt. Rend., **102**, **104**, and **110**; Stohmann, Zeitschr. f. Biologie, **31**; Benedict and Osborne (vegetable proteins), Journ. of biol. Chem., **3**.

The proteins act differently from the fats and carbohydrates. They are only incompletely burnt, and they yield certain decomposition products, which, leaving the body with the excreta, still represent a certain quantity of energy which is lost to the body. The heat of combustion of the proteins is smaller within the organism than outside of it, and they must therefore be specially determined. For this purpose RUBNER[1] fed a dog on washed meat, and he subtracted from the heat of combustion of the food the heat of combustion of the urine and feces, which corresponded to the food taken plus the quantity of heat necessary for the swelling up of the proteins and the solution of the urea. RUBNER has also tried to determine the heat of combustion of the proteins (muscle-proteins) decomposed in the body of rabbits in starvation. According to these investigations, the physiological heat of combustion in calories for each gram of substance is as follows:

1 gram of the dry substance	Calories.
Protein from meat	4.4
Muscle	4.0
Protein in starvation	3.8
Fat (average for various fats)	9.3
Carbohydrates (calculated average)	4.1

The physiological combustion value of the various foods belonging to the same group is not quite the same. It is, for instance, 3.97 calories for a vegetable protein, conglutin, and 4.42 calories for an animal protein body, syntonin. According to RUBNER the normal heat value per 1 gram of animal protein may be considered as 4.23 calories, and of vegetable protein as 3.96 calories. When a person on a mixed diet takes about 60 per cent of the proteins from animal foods and about 40 per cent from vegetable foods, the value of 1 gram of the protein of the food is equivalent to about 4.1 calories. The physiological value of each of the three chief groups of organic foods, by their decomposition in the body, is in round numbers as follows:

	Calories.
1 gram protein	4.1
1 gram fat	9.3
1 gram carbohydrate	4.1
1 gram alcohol	7.1

These figures are generally used in the calculation of the energy content of various foodstuffs and diets.

The extent of gas exchange and the so-called respiratory quotient is, besides the extent of nitrogen elimination, of the greatest importance in the calculation of the extent of energy metabolism and the division of the energy between the protein, fat and carbohydrate.

On comparing the inspired and expired air we learn, on measuring them when dry and at the same temperature and pressure, that the volume

[1] Zeitschr. f. Biologie, 21.

of the expired air is less than that of the inspired air. This depends upon the fact that not all of the oxygen appears again in the expired air as carbon dioxide, because it is not only used in the oxidation of carbon, but also in part in the formation of water, sulphuric acid, and other bodies. The volume of expired carbon dioxide is regularly less than the volume of the inspired oxygen, and the relation $\frac{CO_2}{O}$, which is called the *respiratory quotient*, is generally less than 1.

The magnitude of the respiratory quotient is dependent upon the kind of substances destroyed in the body. In the combustion of pure carbon one volume of oxygen yields one volume of carbon dioxide, and the quotient is therefore equal to 1. The same is true in the burning of carbohydrates, and in the exclusive decomposition of carbohydrates in the animal body the respiratory quotient must be approximately 1. In the exclusive metabolism of proteins it is close to 0.80, and with the decomposition of fat it is 0.7. In starvation, as the animal draws on its own flesh and fat, the respiratory quotient must be a close approach to the latter figure. The respiratory quotient, which is calculated with exclusive combustion of carbohydrate, fat and protein, as respectively, 1, 0.707 and 0.809 and with alcohol is 0.667, also gives important information as to the quality of material decomposed in the body, especially with the supposition that the carbon dioxide elimination is not influenced by some special condition such as a change in the respiratory mechanism. Another supposition is that no incomplete oxidation step in combustion is eliminated.

The respiratory quotient can also be strongly influenced by intermediary processes in the animal body, as by the formation of glycogen from protein, or from fat or by the formation of fat from carbohydrates. In the first case the quotient may be lower than 0.7 and in the last case it can be higher than 1.

Knowledge as to the extent of oxygen consumption is of special importance in the calculation of the energy metabolism from the extent of gas exchange, and one can under some circumstances approximately calculate the energy exchange from the calorific value of the oxygen alone—with regard to the respiratory quotient (ZUNTZ and co-workers). The calorific value of oxygen must be different for each of the three mentioned foodstuffs, as they require different quantities of oxygen for their combustion. For fat and carbohydrate this calorific value can be readily calculated, as these bodies are completely burnt into carbon dioxide and water. One gram of starch uses 828.8 cc. oxygen in its combustion and produces 828.8 cc. carbon dioxide, and 4183 calories of heat are developed. For one liter (=1.43 gram) oxygen, 5047 calories are produced, therefore for every liter (=1.966 gram) carbon dioxide formed,

the same number, 5047 calories, are produced. In an analogous manner the average calorific value of fat for 1 liter of oxygen, 4686 calories, and for 1 liter carbon dioxide, 6629 calories, can be calculated.

These figures, which represent the physiological combustion values per 1 gram of food-stuffs, derived from the carbon dioxide output or the oxygen in-take in (grams or) liters which are represented by the quotients $\frac{\text{Cal.}}{\text{L.CO}_2}$ or $\frac{\text{Cal.}}{\text{L.O}_2}$, have been called the *calorific coefficients*.

With proteins, because of the unequal composition of the different proteins, the results are uncertain and variable, and the calculation is much more complicated. As example we will give the following calculation of ZUNTZ [1] for the fat-free dry substance of meat.

This substance consisted in 100 parts

	52.38g.C.;	7.27g.H.;	22.68g.O.;	16.65g.N.;	1.02g.S.
Of which were found in the urine.	9.406	2.663	14.099	16.28	1.02
Of which were found in the feces.	1.471	0.212	0.889	0.37	
Retained.....	41.50C;	4.40H;	7.69O;	0.0N;	0.0S.

From this residue, with the taking up of 96.63 liters of oxygen, besides 39.6 grams water, 77.39 liters carbon dioxide were formed and the respiratory quotient is therefore 0.801. Now 100 grams of such dry meat substance on complete combustion yields 563.09 calories, and if we subtract the calorific value of the corresponding urine (=113.70 calories) and feces (=17.76 calories), the sum, 131.46 calories, then 431.63 calories were set free in the body. For every gram of nitrogen eliminated in the urine (16.28 gram) there is produced $\frac{431.63}{16.28}=26.51$ calories; the corresponding quantity of oxygen is $\frac{96.63}{16.28}=5.91$ liter O and the corresponding quantity of CO_2 produced is $\frac{77.39}{16.28}=4.75$ liters CO_2. The calorific value for 1 liter of oxygen consumed is therefore $\frac{26.51}{5.91}=4.485$ calories, and for 1 liter of carbon dioxide produced $\frac{26.51}{4.75}=5.579$ calories.

For milk protein ZUNTZ has calculated for 1 gram urea nitrogen 5.8 liters oxygen, 4.6 liters carbon dioxide and 27 calories. The calorific value can be calculated from this for 1 liter O = 4.66 and for 1 liter CO_2 = 5.87 calories. If we take the average of these calculations we obtain the calorific coefficients $\frac{\text{Cal.}}{\text{L.O}_2}=4.57$ and $\frac{\text{Cal.}}{\text{L.CO}_2}=5.73$ for protein.

For the three foodstuffs we have the following calorific values:

	Per 1 liter Oxygen.	Relative value.	Per 1 liter Carbon dioxide.	Relative value.
Protein.....	4.57	100	5.73	113.4
Fat.....	4.69	102.6	6.63	131.3
Carbohydrate.....	5.05	110.5	5.05	100.0

[1] Zuntz, Loewy, Müller and Caspari, Höhenklima und Bergwanderungen, Berlin, 1906, pages 102, 103.

The figures for the oxygen vary less than those for the carbon dioxide, and this is a reason why the oxygen values are better suited than the CO_2 values for calculating the energy production from the extent of gas exchange. Other investigators have obtained results which correspond more or less with the above values for the heat value of oxygen, and E. VOIT and KUMMACHER,[1] who have made calculations in another way, have obtained still smaller differences for the relative oxygen value.

From what was said above we can calculate the extent of protein metabolism, the corresponding development of energy and the corresponding absorption of oxygen and carbon dioxide formation, from the quantity of nitrogen in the urine. If we subtract the oxygen and carbon dioxide values from the total, directly determined gas exchange, the result represents the fats and carbohydrates used. According to ZUNTZ from this residue we can calculate the heat value of the oxygen used as well as the division of the decomposition of the fat and carbohydrate by considering the respiratory quotient. For this purpose ZUNTZ and SCHUMBURG have constructed a table, an abstract of which we give below, taken from the work of MAGNUS-LEVY.[2]

R. Q.	Calories value per 1 liter O.	Division in per cent.	
		Carbohydrate.	Fat.
1.000	5.047	100	0
0.950	4.986	83	17
0.900	4.924	66	34
0.850	4.863	49	51
0.800	4.801	32	68
0.750	4.740	15	85
0.707	4.686	0	100

As the calorific oxygen values in the combustion of protein, fat and carbohydrate show no great differences among themselves, in those cases where, as in starvation, the part taken by the proteins in the total metabolism is relatively small, one can calculate the total energy exchange, without any striking error, from the respiratory quotient and the oxygen used. This is especially important in experiments of short duration where the protein metabolism cannot be directly determined, but is calculated from the nitrogen elimination occurring during a longer time. The method of ZUNTZ and GEPPERT, mentioned on page 869, has shown itself especially useful in the study of the material and force exchange in these experiments of short duration, while the respiration apparatus constructed on PETTENKOFER'S or the REGNAULT-REISET principle are only useful in experiments over a longer period.

KAUFMANN [3] incloses the individual to be experimented upon in a capacious sheet-iron room, which serves both as a respiration-chamber and a calorimeter,

[1] Voit, Zeitschr. f. Biol., 44; Kummacher, *ibid.*

[2] A. Magnus-Levy in v. Noorden's Handb. d. Pathol. des Stoffwechsels, Bd. 1. (1906).

[3] Arch. d. Physiologie (5), 8.

and which permits the estimation of the nitrogen of the urine and the carbon dioxide expired, as well as the inspired oxygen and the quantity of heat produced. If we start from the theoretically calculated formulæ for the various possible transformations of the proteins, fats, and carbohydrates in the body, it is clear that other values must be obtained for the heat, carbon dioxide, oxygen, and nitrogen of the urine, when one, for example, admits of a complete combustion of proteins to urea, carbon dioxide, and water, or of a partial splitting off of fat. Another relation between heat, carbon dioxide, and oxygen is also to be expected when the fat is completely burnt or when it is decomposed into sugar, carbon dioxide, and water. In this way, by a comparison of the values found in special cases with the figures calculated for the various transformations, KAUFMANN attempts to explain the various decomposition processes in the body under different nutritive conditions.

The organic foodstuffs serve in part to replace the necessary losses of the organs and in part as sources of energy. Under all circumstances a restitution of the protein-like constituents of the organs is necessary. This replacement is, according to RUBNER, represented by the so-called *wear-and-tear quota* (see below) which amounts to about 4–6 per cent of the total energy transformed and which can be supplied by proteins only. For the supply of the remaining exchange, which according to RUBNER serves as source of energy, all three groups of organic foodstuffs can be used, and investigations carried out by RUBNER have taught that these foodstuffs can act as sources of energy in the animal body in a proportion which corresponds with the respective figures of their heat value. This is apparent from the following table. In this is found the weight of the various foods equal to 100 grams of fat, a part determined from experiments on animals and a part calculated from figures of the heat values:

	From Experiments on Animals.	From the Heat Value.	Difference, per cent.
Syntonin	225	213	+5.6
Muscle-flesh (dried)	243	235	+4.3
Starch	232	229	+1.3
Cane-sugar	234	235	−0
Glucose	256	255	−0

From the given *isodynamic value* of the various foods it follows that these substances replace one another in the body almost in exact ratio to the energy contained in them. Thus in round numbers 227 grams of protein and carbohydrate are equal to or isodynamic with 100 grams of fat in regard to source of energy, because each yields 930 calories on combustion in the body.

By means of recent very important calorimetric investigations, RUBNER [1] has shown that the heat produced in an animal in several series of experiments extending over forty-five days corresponded to within 0.47 per cent of the physiological heat of combustion calculated from the decom-

[1] Zeitschr. f. Biologie, 20.

posed body and foods. ATWATER and his collaborators [1] have made some very thorough investigations on this subject on men. In their experiments they made use of a large respiration calorimeter, which not only exactly determined the excreta, but also made a calorimetric determination of the heat given out by the person experimented upon, i.e., the work performed. From the results of these experiments they found an almost absolutely complete agreement between the calories found directly and those calculated.

This isodynamic law is of fundamental value in the study of metabolism and nutrition. The quantity of energy in the transformed foods or the constituents of the body may be used as a measure for the total consumption of energy, and the knowledge of the quantity of energy in the foods must also be the basis for the calculation of dietaries for human beings under various conditions.

The isodynamic theory has been accepted by a large number of investigators, but not by all. Certain of them, especially the French, accept an *isoglucosic* instead of the isodynamic. According to this theory the organism for its physiological functions can use glucose only, and as a formation of glucose is possible from proteins as well as fats, those quantities of food-stuffs are to be considered as equivalent which yield an equal amount of glucose.

The heat value of a foodstuff can be directly determined in a calorimeter, but may also be calculated from its composition. If one subtracts from the gross heat value of the food obtained in one way or another the combustion heat of the feces and urine with the same diet, there is obtained the net calorific value of the diet. This value, calculated in percentage of the total energy content of the food, is called the *physiological availability* by RUBNER.[2] In order to elucidate this we will give a few of RUBNER'S values. The loss in calories, as well as the physiological availability, is calculated in percentages of the total energy content of the food.

Food.	Loss in per cent.		Total loss in per cent.	Availability in per cent.
	In Urine.	In the Feces.		
Cow's milk	5.13	5.07	10.20	89.8
Mixed diet (rich in fat)	3.87	5.73	9.60	90.4
Mixed diet (poor in fat)	4.70	6.00	10.70	89.3
Potatoes	2.00	5.60	7.60	92.4
Graham bread	2.40	15.50	17.90	82.1
Rye bread	2.20	24.30	26.50	73.5
Meat	16.30	6.90	23.20	76.8

In order to simplify the calculation of the energy exchange there exist other standard factors, besides the above-mentioned standard figures for the physiological

[1] Bull. of Dept. of Agric., Washington, **44**, **63**, **69**, and **109**, and Ergebnisse der Physiologie, **3**.

[2] Zeitschr. f. Biologie, **42**.

calorific value of the organic foodstuffs, also for the carbon of the carbon dioxide, and for the oxygen. Thus for 1 gram of meat (dry substance) free from fat and extractives we have the calculated value of 5.44–5.77 calories. KÖHLER [1] found 5.678 calories for 1 gram of ash and fat-free dried-meat substance of the ox and 5.599 calories for horse meat. According to FRENTZEL and SCHREUER [2] 45.4 calories is calculated for 1 gram of nitrogen in fat and ash-free dried-meat feces (dog), while 6.97 to 7.45 calories is calculated for 1 gram of nitrogen in meat-urine. The calorific urine quotient $\frac{\text{calories}}{N}$ seems still, as above given, not to be constant for human beings, but is dependent upon the variety of food.

II. METABOLISM IN STARVATION AND WITH INSUFFICIENT NUTRITION.

In starvation the decomposition in the body continues uninterruptedly, though with decreased intensity; but, as it takes place at the expense of the substance of the body, it can continue for a limited time only. When an animal has lost a certain fraction of the mass of the body, death is the result. This fraction varies with the condition of the body at the beginning of the starvation period. Fat animals succumb when the weight of the body has sunk to one-half of the original weight. Otherwise, according to CHOSSAT,[3] animals die as a rule when the weight of the body has sunk to two-fifths of the original weight. The period when death occurs from starvation not only varies with the varied nutritive condition at the beginning of starvation, but also with the more or less active exchange of material. This is more active in small and young animals than in large and older ones, but different classes of animals show an unequal activity. Children succumb in starvation in 3–5 days after having lost one-fourth of their body mass. Grown persons may, as observed upon SUCCI,[4] and other professional fasters, starve for twenty days or more without lasting injury; and there are reports of cases of starvation extending over a period of even more than forty to fifty days. Dogs may starve, according to several observers, 50–60 days. HAWK [5] and co-workers have recorded a case where a dog was starved for 117 days and lost about 63 per cent of its original weight. Snakes and frogs can starve for one-half a year or even a whole year.

In starvation the *weight of the body* decreases. The loss of weight is greatest in the first few days, and then decreases rather uniformly. In small animals the absolute loss of weight per day is naturally less than in larger animals. The relative loss of weight—that is, the loss of weight

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[1] Zeitschr. f. physiol. Chem., **31**.

[2] The works of Frentzel and Schreuer may be found in Arch. f. (Anat. u.) Physiol., 1901, 1902, and 1903.

[3] Cited from Voit in Hermann's Handbuch, **6**, Thl. 1, 100.

[4] See Luciani, Das Hungern. Hamburg u. Leipzig, 1890.

[5] P. B. Hawk, P. E. Howe, and H. A. Mattil, Journ. of biol. Chem., **11**.

of the unit of the weight of the body, namely, 1 kilo—is, on the contrary, greater in small animals than in larger ones. The reason for this is that the smaller animals have a greater surface of body in proportion to their mass than larger animals, and the greater loss of heat caused thereby must be replaced by a more active consumption of material.

It follows from the decrease in the weight of the body that the absolute extent of metabolism must diminish in starvation. If, on the contrary, the extent of metabolism is referred to the unit of weight of the body, namely, 1 kilo, it appears that this quantity remains almost unchanged during starvation. The investigations of ZUNTZ, LEHMANN, and others,[1] on the professional faster CETTI, showed on the third and sixth days of starvation an average consumption of 4.65 cc. oxygen per kilo in one minute, and on the ninth to eleventh day an average of 4.73 cc. The calories, as a measure of the metabolism, fell on the first to fifth day of starvation from 1850 to 1600 calories, or from 32.4 to 30 per kilo, and it remained nearly unchanged, if referred to the unit of body weight.[2] In man the average daily energy consumption in starvation amounts to about 30–32 calories per kilo.

The *extent of the metabolism of proteins*, or the elimination of nitrogen by the urine, which is a measure of the same, diminishes as the weight of the body diminishes. This decrease is not regular or the same during the entire period of starvation, and the extent depends, as the experiments made upon carnivora have shown, upon several circumstances. During the first few days of starvation the excretion of nitrogen is greatest, and the richer the body is in protein, due to the food previously taken, the greater is the protein catabolism or the nitrogen elimination, according to VOIT. The nitrogen elimination diminishes the more rapidly—that is, the curve of the decrease is more sudden—the richer in proteins the food was which was taken before starvation. This condition is apparent from the following table of data of three different starvation experiments made by VOIT[3] on the same dog. This dog received 2500 grams of meat daily before the first series of experiments, 1500 grams of meat daily before the second series, and a mixed diet relatively poor in nitrogen before the third series.

Day of Starvation.	Grams of Urea Eliminated in Twenty-four Hours.		
	Ser. I.	Ser. II.	Ser. III.
First	60.1	26.5	13.8
Second	24.9	18.6	11.5
Third	19.1	15.7	10.2
Fourth	17.3	14.9	12.2
Fifth	12.3	14.8	12.1
Sixth	13.3	12.8	12.6
Seventh	12.5	12.9	11.3
Eighth	10.1	12.1	10.7

[1] Berlin. klin. Wochenschr., 1887.

[2] See also Tigerstedt and collaborators in Skand. Arch. f. Physiol., 7.

[3] See Hermann's Handbuch, 6, Thl. 1, 89.

In man and also in animals sometimes a rise in the nitrogen excretion is observed about the second or third starvation day, which is then followed by a regular diminution. This rise is explained by PRAUSNITZ, TIGERSTEDT, LANDERGREN,[1] as follows: At the commencement of starvation the protein metabolism is reduced by the glycogen still present in the body. After the consumption of the glycogen, which takes place in great part during the first days of starvation, the destruction of proteins increases as the glycogen action decreases, and then decreases again when the body has become poorer in available proteins.

Other conditions, such as varying quantities of fat in the body, have an influence on the rapidity with which the nitrogen is eliminated during the first days of starvation. After the first few days of starvation the elimination of nitrogen is more uniform. It may diminish gradually and regularly until the death of the animals or there may be a rise in the last days, a so-called premortal increase. Whether the one or the other occurs depends upon the relation between the protein and fat content of the body.

Like the destruction of proteins during starvation, the *decomposition of fat* proceeds uninterruptedly, and the greatest part of the calories needed during starvation are supplied by the fats. According to RUBNER and VOIT the protein catabolism varies only slightly in starving animals at rest and at an average temperature, and forms a constant portion of the total exchange of energy; of the total calories in dogs 10–16 per cent comes from the protein decomposition and 84–90 per cent from the fats. This is at least true for starving animals which had a sufficiently great original fat content. If on account of starvation the animal has become relatively poorer in fat and the fat content of the body has fallen below a certain limit, then in order to supply the calories necessary, a larger quantity of protein is destroyed and the premortal increase now occurs (E. VOIT). The reason for this premortal rise in protein catabolism is still not completely understood (SCHULZ and collaborators[2]).

Since the fat has a diminishing influence on the destruction of proteins corresponding to what was said above, the elimination of nitrogen in starvation is less in fat than in lean individuals. For instance, only 9 grams of urea were voided in twenty-four hours during the later stages of starvation by a well-nourished and fat person suffering from disease of the brain, while I. MUNK found that 20–29 grams urea were voided daily by CETTI,[3] who had been poorly nourished.

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[1] Prausnitz, Zeitschr. f. Biologie, **29**; Tigerstedt and collaborators, l. c.; Landergren, Undersökningar öfver menniskans ägghviteomsättning, Inaug.-Diss. Stockholm, 1902.

[2] Voit, Zeitschr. f. Biologie, **41**; 167 and 502. See also Kaufmann, *ibid.*, and N. Schulz, *ibid.*, and Pflüger's Arch., **76**, with Mangold, Stübel and Hempel, *ibid.*, **114**.

[3] Berl. klin. Wocheuschr., 1887.

The investigations on the *exchange of gas* in starvation have shown, as previously mentioned, that its absolute extent is diminished, but that when the consumption of oxygen and elimination of carbon dioxide are calculated on the unit weight of the body, 1 kilo, this quantity quickly sinks to a minimum and then remains unchanged, or, on the continuation of the starvation, may actually rise. It is a well-known fact that the body temperature of starving animals remains almost constant, without showing any appreciable decrease, during the greater part of the starvation period. The temperature of the animal first sinks a few days before death, which occurs at about 33–30° C.

From what has been said about the respiratory quotient it follows that in starvation it is about the same as with fat and meat exclusively as food, i.e., approximately 0.7. This is often the case, but it may occasionally be lower, 0.65–0.50, as observed in the cases of CETTI and SUCCI. This can be explained by an elimination of acetone bodies by the urine; a part can be accounted for perhaps by a formation and deposition of glycogen from protein.

Water passes uninterruptedly from the body in starvation even when none is taken. If the quantity of water in the tissues rich in proteins is considered as 70–80 per cent, and the quantity of proteins in them 20 per cent, then for each gram of protein destroyed about 4 grams of water are set free. This liberation of water from the tissues is generally sufficient to supply the loss of water, and starvation is ordinarily not accompanied with thirst.

The loss of water calculated on the percentage of the total organism must naturally be essentially dependent upon the previous amount of fatty tissue in the body. In certain cases the starving animal body has indeed been found richer in water; but if we bear these conditions in mind, then, it seems, according to BÖHTLINGK,[1] that, from experiments upon white mice, the animal body is poorer in water during inanition. The body loses more water than is set free by the destruction of the tissues.

The *mineral substances* leave the body uninterruptedly in starvation until death, and the influence of the destruction of tissues is plainly perceptible by their elimination. Because of the destruction of tissues rich in potassium the proportion between potassium and sodium in the urine changes in starvation, so that, contrary to the normal conditions, the potassium is eliminated in proportionately greater quantities.

Contrary to the above BÖHTLINGK with starving white mice, and KATSUYAMA [2] with starving rabbits found a greater excretion of sodium than potassium.

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[1] Arch. des sciences biol. de St. Pétersbourg, 5.

[2] Böhtlingk, l. c.; Katsuyama, Zeitschr. f. physiol. Chem., 26.

MUNK observed, in CETTI'S case, an increase in the elimination of phosphoric acid in relation to the *N*-elimination, which indicates an increased decomposition of bone-substance, and this explanation is supported by the fact that a simultaneous increase in the elimination of lime and magnesia occurs. Recently WELLMANN [1] showed that in rabbits, the increase in the elimination of phosphorus, calcium and magnesium in starvation corresponds to the loss in the bones of these constituents.

The question as to the participation of the different organs in the loss of weight of the body during starvation is of special interest. In elucidation of this point we give the following results of CHOSSAT'S experiments on pigeons, and those of VOIT [2] on a male cat. The results are percentages of weight lost from the original weight of the organ.

	Pigeon (CHOSSAT).	Male Cat (VOIT).
Adipose tissue	93 per cent.	97 per cent.
Spleen	71 "	67 "
Pancreas	64 "	17 "
Liver	52 "	54 "
Heart	45 "	3 "
Intestine	42 "	18 "
Muscles	42 "	31 "
Testicles	— "	40 "
Skin	33 "	21 "
Kidneys	32 "	26 "
Lungs	22 "	18 "
Bones	17 "	4 "
Nervous system	2 "	3

The total quantity of blood, as well as the quantity of solids contained therein, decreases, as PANUM and others [3] have shown, in the same proportion as the weight of the body. Concerning the loss of water by different organs authorities disagree, LUKJANOW [4] claiming that the various organs differ from each other in this respect.

The above-tabulated results cannot serve as a measure of the metabolism in the various organs during starvation. For instance, the nervous system shows only a small loss of weight as compared with the other organs, but from this it must not be concluded that the exchange of material in this system of organs is least active. The conditions may be quite different; for one organ may derive its nutriment during starvation from some other organ and exist at its expense. A positive conclusion cannot be drawn in regard to the activity of the metabolism in an organ from the loss of weight of that organ in starvation. Death by starvation is not the result of the death of all the organs of the body,

[1] Munk, Berl. klin. Wochenschr., 1887; Wellmann, Pflüger's Arch., 121.
[2] Cited from Voit in Hermann's Handbuch, 6, Part I, 96 and 97.
[3] Panum, Virchow's Arch., 29; London, Arch. d. scienc. biol. de St. Pétersbourg, 4.
[4] Zeitschr. f. physiol. Chem., 13.

but it depends more likely upon the disturbance in the nutrition of a few less vitally important organs (E. VOIT [1]).

In calculating or determining the loss of weight of the organs in starvation the original fat content of the organs must be considered. With the consideration of the fat content of the organs, determined or estimated in a special way before the starvation period and at the end, E. VOIT [2] found the following loss of weight in the supposed fat-free organs in starvation, namely, muscles 41 per cent, viscera 42 per cent, skin 28 per cent, and skeleton 5 per cent.

The quantity of urine nitrogen sinks in starvation corresponding to the protein catabolism, but to a varying degree in different individuals. The lowest value observed thus far in man was 2.82 grams per diem as found by E. and O. FREUND on the twenty-first day in the faster SUCCI. Calculated on 1 kilogram of body weight, the urine nitrogen, as is to be expected, shows striking differences in different persons; in CETTI and SUCCI it was 0.150–0.200 gram on the fifth to tenth day of starvation. The division of the nitrogen in the urine in starvation is unlike that in the normal condition. The relative amount of urea diminishes, as shown by E. and O. FREUND, BRUGSCH and CATHCART,[3] so that instead of being about 85 per cent of the total nitrogen under normal conditions it can sink to 54 per cent (BRUGSCH). At the same time because of the abundant formation of acetone bodies (starvation acidosis) the quantity of ammonia increases considerably (BRUGSCH, CATHCART). A relative increase in the neutral sulphur of the urine also takes place (BENEDICT, CATHCART [4]). Creatine also occurs in starvation urine and according to HAWK [5] and co-workers the elimination of creatine is much greater than the creatinine a few days before the premortal nitrogen elimination.

One must differentiate between the real starvation metabolism and the metabolism in the inanition condition, the *basal requirement* (MAGNUS-LEVY) or the *maintenance value* (LOEWY [6]). With this we understand the metabolism in uniform, medium temperature, with absolute bodily rest and inactivity of the intestinal canal. As a measure of this we determine the gas exchange in a person lying down with as perfect complete muscular rest as possible, or sleeping in the early morning and at least twelve hours after a light meal not rich in carbohydrates. This

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[1] Zeitschr. f. Biologie, **41**.

[2] *Ibid.*, **46**.

[3] E. and O. Freund, Wien. klin. Rundschau, 1901, Nos. 5 and 6; Brugsch, Zeitschr. f. exp. Path. u. Therap., **1** and **3**; Cathcart, Bioch. Zeitschr., **6**.

[4] Zeitschr. f. klin. Med., **36**; Cathcart, l. c.

[5] Journ. of biol. Chem., **11**.

[6] Magnus-Levy in v. Noorden's Handbuch, and Loewy in Oppenheimer's Handbuch d. Biochemie, Bd. **4**.

basal requirement is the measure of the energy necessary for the performance of all the functions necessary to maintain life during rest; and all work above this minimum activity is called *productive increase* by MAGNUS-LEVY. The basal requirement is almost constant for the same individual and serves as the starting point in the study of the action of different influences such as work, food, diseased conditions, etc., upon metabolism. The extent of this basal requirement, as determined by the gas exchange according to the ZUNTZ-GEPPERT method, and by JOHANSSON[1] and collaborators amounts in men of 60–70 kilos body weight to about 220–250 cc. oxygen and 160–200 cc. carbon dioxide per minute, which equals 20–24 grams carbon dioxide per hour. JOHANSSON found in forced complete muscular rest 20.7 grams CO_2 per hour and 24.8 grams CO_2 in the ordinary resting. GIGON[2] found about 23.4 grams CO_2 and 21 grams oxygen for the basal requirement. According to MAGNUS-LEVY the total daily metabolism can be calculated for the basal requirement as 1625 calories, or including the rise due to the partaking of food as 1800 calories. According to GIGON the basal requirement consists of 15.22 per cent proteïn, 15–35.2 per cent carbohydrates and 44.5–70 per cent fat.

The food may be quantitatively insufficient, and the final result of this is absolute inanition. The food may also be qualitatively insufficient or, as we say, inadequate. This occurs when any of the necessary nutritive bodies are absent in the food, while the others occur in sufficient or perhaps even in excessive amounts.

Lack of Water in the Food. The quantity of water in the organism is greatest during fœtal life and then decreases with increasing age. Naturally, the quantity differs essentially in different organs. The enamel, with only 2 p. m. water, is the tissue poorest in water, while the teeth contain about 100 p. m. and the fatty tissue 60–120 p. m. water. The bones, with 140–440 p. m., and the cartilage with 540–740 p. m. are somewhat richer in water, while the muscles, blood and glands, with 750 to more than 800 p. m., are still richer. The quantity of water is even greater in the animal fluids (see preceding chapter), and the adult body contains in all about 630 p. m. water.[3] It follows from what has been given in Chapter I in regard to the great importance of water for living processes, that if the loss of water is not replaced by fresh supply, the organism must succumb sooner or later. Death occurs indeed sooner from lack of water than from complete inanition (LANDAUER, NOTHWANG).

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[1] The literature can be found in the works of Magnus-Levy and Loewy.

[2] Johansson, Skand. Arch. f. Physiol., 7, 8, 21, and Nord. Med. Arch. Festband, 1897; see also Magnus-Levy; Gigon, Pflüger's Arch., 140.

[3] See Voit, in Hermann's Handbuch, 6, part 1, 345.

If water is withdrawn for a certain time, as LANDAUER and especially STRAUB have shown, it has an accelerating influence upon the decomposition of protein. This increased destruction has, according to LANDAUER, the purpose of replacing a part of the water removed, by the production of water by means of the increased metabolism. The deprivation of water for a short time may, according to SPIEGLER,[1] especially in man, cause a diminution in the protein metabolism by means of a reduced protein absorption.

Lack of Mineral Substances in the Food. In the previous chapters attention has repeatedly been called to the importance of the mineral bodies and also to the occurrence of certain mineral substances in certain amounts in the various organs. The mineral content of the tissues and fluids is not very great as a rule. With the exception of the skeleton, which contains as average about 220 p. m. mineral bodies (VOLKMANN[2]), the animal fluids or tissues are poor in inorganic constituents, and the quantity of these amounts as a rule, only to about 10 p. m. Of the total quantity of mineral substances in the organism, the greatest part occurs in the skeleton, 830 p. m., and the next greatest in the muscles, about 100 p. m. (VOLKMANN).

The mineral bodies seem to be partly dissolved in the fluids and partly combined with organic substances, but nothing definite can be given as to the kind of combination, or whether they occur in stoichiometric proportions, or whether they are simply adsorption combinations. In accordance with this the organism persistently retains, with food poor in salts, a part of the mineral substances, also such as are soluble, as the chlorides. On the burning of the organic substances the mineral bodies combined therewith are set free and may be eliminated. It is also admitted that they in part combine with the new products of the combustion, and in part with organic nutritive bodies poor in salts or nearly salt-free, which are absorbed from the intestinal canal and are thus retained (VOIT, FORSTER[3]).

If this statement is correct, it is possible that a constant supply of mineral substances with the food is not absolutely necessary, and that the amount of inorganic bodies which must be administered is insignificant. The question whether this is so or not has not, especially in man, been sufficiently investigated; but generally we consider the need of mineral

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[1] Landauer, Maly's Jahresber., **24**; Nothwang, Arch. f. Hyg., 1892; Straub, Zeitschr. f. Biol., **37** and **38**; Spiegler, *ibid.*, **41**.

[2] See Hermann's Handbuch., **6**, pt. 1, 353.

[3] Forster, Zeitschr. f. Biologie, **9**. See also Voit, in Hermann's Handbuch, **6**, Part 1, 354. In regard to the occurrence and the behavior of the various mineral constituents of the animal body see the work of Albu and Neuberg, Physiologie und Pathologie des Mineralstoffwechsel, Berlin, 1906.

substances by man as very small. It may, however, be assumed that man usually takes with his food a considerable excess of mineral substances.

Experiments to determine the results of an insufficient supply of mineral substances with the food in animals have been made by several investigators, especially FORSTER. He observed, on experimenting with dogs and pigeons with food as poor as possible in mineral substances, that a very suggestive disturbance of the functions of the organs, particularly the muscles and the nervous system, appeared, and that death resulted in a short time, earlier in fact than in complete starvation. On observations made upon himself, TAYLOR[1] found on partaking less than 0.1 gram salts *per diem* that the chief disturbance occurred in the muscular system.

BUNGE in opposition to these observations of FORSTER'S has suggested that the early death of these cases was not caused by the lack of mineral salts, but more likely by the lack of bases necessary to neutralize the sulphuric acid formed in the combustion of the proteins in the organism; these bases must then be taken from the tissues. In accordance with this view, BUNGE and LUNIN[2] also found, in experimenting with mice, that animals which received nearly ash-free food with the addition of sodium carbonate were kept alive twice as long as those which had the same food without the sodium carbonate. Special experiments also show that the carbonate cannot be replaced by an equivalent amount of sodium chloride, and that to all appearances it acts by combining with the acids formed in the body. The addition of alkali carbonate to the otherwise nearly ash-free food may indeed delay death, but cannot prevent it, and even in the presence of the necessary amount of bases death results from lack of mineral substances in the food.

With an insufficient supply of *chlorides* with the food the elimination of chlorine by the urine decreases constantly, and at last it may stop entirely, while the tissues still persistently retain the chlorides. It has already been stated (Chapter VIII) how chloride starvation influences other functions, especially the secretion of gastric juice. If there be a lack of sodium as compared with potassium, or if there be an excess of potassium compounds in any other form than KCl, the potassium combinations are replaced in the organism by NaCl, so that new potassium and sodium compounds are produced which are voided with the urine. The organism becomes poorer in NaCl, which therefore must be taken in greater amounts from the outside (BUNGE). This occurs continuously

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[1] University of California Publications, Pathol., 1.

[2] Bunge, Lehrbuch der physiol. Chem., 4. Aufl., 97; Lunin, Zeitschr. f. physiol. Chem., 5.

in herbivora, and in man with vegetable food rich in potash. For human beings, and especially for the poorer classes of people who live chiefly on potatoes and foods rich in potash, common salt is not only a condiment, but a necessary addition to the food (BUNGE [1]). On the behavior of chlorides, especially sodium chloride, in the animal body as well as the elimination or the retention of NaCl in diseases, we have an abundance of investigations, which may be found in ALBU and NEUBERG'S [2] work, previously cited.

Lack of Alkali Carbonates or Bases in the Food. The chemical processes in the organism are dependent upon the presence in the tissues and tissue-fluids of a certain reaction, and this reaction, which is habitually alkaline toward litmus and neutral toward phenolphthalein, is chiefly due to the presence of alkali carbonates and carbon dioxide and in a lesser degree to alkali phosphates. The alkali carbonates are also of great importance, not only as a solvent for certain protein bodies and as constituents of certain secretions, such as the pancreatic and intestinal juices, but they are also a means of transportation of the carbon dioxide in the blood. It is therefore easy to understand that a decrease below a certain point in the quantity of alkali carbonate must endanger life. Such a decrease not only occurs with lack of bases in the food which brings about various disturbances and death by a relatively great production of acids through the burning of the proteins, but it also occurs when an animal is given dilute mineral acids for a period. The importance of ammonia as a means of neutralizing the acids produced or introduced into the body as well as the unequal resistance of man and other animals toward this action of acids has already been discussed in Chapter XIV.

Lack of Phosphates and Earths. With the exception of the value of the alkaline earths as carbonates and more especially as phosphates in the physical composition of certain structures, such as the bones and teeth, their physiological importance is almost unknown. The importance of calcium for certain enzymotic processes and of calcium ions for the functions of the muscles, and especially for cell life, gives an indication of the necessity of the alkaline earths to the animal organism. Little is known of the need of these earth in adults, and no average results can be given. According to KOCHMANN and PETZSCH [3] we cannot conceive of a certain calcium minimum (in dogs) as the Ca needs vary with different foods. With a Ca equilibrium we can cause an increased elimination of calcium by increasing the quantity of protein, of fat, or of carbohydrate in the food and this probably depends upon a giving up

[1] Zeitschr. f. Biologie, 9.

[2] See footnote 3, page 899.

[3] Kochmann, Bioch. Zeitschr., 31, with Petzsch, *ibid.*, 32.

of calcium phosphate by the skeleton. It is impossible to give positive figures for the need of phosphates or phosphoric acid, whose value is recognized not only in the construction of the bones, but also in the functions of the muscles, the nervous system, the glands, the organs of generation, etc. The extent of this need is most difficult to determine, as the body shows a strong tendency, when increased amounts of phosphorus are introduced, to retain more than is necessary. The need of phosphates, which, according to EHRSTRÖM,[1] corresponds in adults to a minimum of 1 to 2 grams phosphorus, is relatively smaller in adults than in young, developing animals, and in these latter the question of the result of an insufficient supply of earthy phosphates and alkaline earths upon the bone tissue is of special interest. For details we refer to Chapter IX and to the cited work of ALBU-NEUBERG.

Another important question is, How far do the phosphates take part in the construction of the phosphorized constituents of the body or to what extent are they necessary? The experiments of RÖHMANN and his pupils[2] with phosphorized (casein, vitellin) and non-phosphorized proteins (edestin) and phosphates show that with the introduction of casein and vitellin a deposition of nitrogen and phosphorus takes place, while with non-phosphorized protein and phosphates this does not seem to occur. The body apparently does not have the power of building up the phosphorized cell constituents necessary for cell life from non-phosphorized proteins and phosphates. On the contrary, according to the observations of several investigators, the lecithins seem to possess this power. As known from the investigations of MEISCHER, the development of the generative organs of the salmon, which are very rich in nuclein substances and phosphatides, from the muscles which are relatively poor in organic-combined phosphorus, seem to indicate a synthesis of phosphorized organic substance from the phosphates. The investigations of HART, MCCOLLUM and FULLER,[3] who found that pigs with food poor in phosphorus develop just as well with inorganic phosphates as with organic phosphorus compounds, also indicate such a formation. The recent investigations of MCCOLLUM[4] on rats show that these animals can take up the entire need of phosphorus for the skeleton as well as for the reformation of nucleins and phosphatides in the form of inorganic

[1] Skand. Arch. f. Physiol., 14.

[2] The literature on feeding experiments with phosphorized and non-phosphorized food can be found in McCollum, Amer. Journ. of Physiol., 25.

[3] Hart, McCollum and Fuller, Amer. Journ., of Physiol., 23. See also Lipschütz, Pflüger's Arch., 143. The literature on the phosphorus metabolism can also be found in Albu and Neuberg, Physiologie und Pathologie des Mineralstoffwechsel, Berlin, 1906.

[4] Amer. Journ. of Physiol., 25.

phosphorus. Also the investigations of v. WENDT and HOLSTI[1] show that a synthesis of organic phosphorized substances from phosphates is very probable. The feeding experiments of OSBORNE and collaborators, which we will soon discuss, and which extend over a long period where the animals were fed with proteins, fat, carbohydrates and mineral substances free from phosphorus, give especially strong proof of the ability of the animal to construct phosphatides and nucleins from only inorganic phosphorus.

Lack of Iron. As iron is an integral constituent of hæmoglobin, absolutely necessary for the supply of oxygen, it is an indispensable constituent of food. Iron is a never-failing constituent of the nucleins and nucleoproteins, and herein lies another reason for the necessity of the introduction of iron. Iron is also of great importance in the action of certain enzymes, the oxidases. In iron starvation, iron is continually eliminated, even though in diminished amounts; and with an insufficient supply of iron with the food the formation of hæmoglobin decreases. The formation of hæmoglobin is not only enhanced by the supply of organic iron, but also, according to the general view, by inorganic iron preparations. The various divergent reports of this question have already been given in a previous chapter (on the blood).

In the absence of *protein bodies* in the food the organism must nourish itself by its own protein substances, and with such nutrition it must sooner or later succumb. By the exclusive administration of fat and carbohydrates the consumption of proteins in these cases is very considerably reduced. For a long time we believed in the view suggested by C. and E. VOIT[2] that with a nitrogen-free diet the protein metabolism could never be reduced to as small a value as in starvation, but now, due to the investigations of HIRSCHFELD, KUMAGAWA, KLEMPERER, SIVÉN, LANDERGREN and recently those of THOMAS,[3] we learn that the protein metabolism with such a diet can be smaller than in complete starvation. With exclusive feeding of sugar, according to THOMAS, the nitrogen elimination can be reduced in a few days to the wear and tear quota, and he has observed an elimination of only 30 milligrams nitrogen per day and per kilo of body weight.

The absence of *fats* and *carbohydrates* in the food affects carnivora and herbivora somewhat differently. It is not known whether carnivora

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[1] v. Wendt, Skand. Arch. f. Physiol., **17**; Holsti; *ibid.*, **23**. See also Gregersen, Zeitschr. f. physiol. Chem., **71**.

[2] Zeitschr. f. Biologie, **32**.

[3] Hirschfeld, Virchow's Arch., **114**; Kumagawa, *ibid.*, **116**; Klemperer, Zeitschr. f. klin. Med., **16**; Sivén, Skand. Arch. f. Physiol., **10** and **11**; Landergren, l. c., **11**; footnote 1, page 894, also Maly's Jahresber., **32**; Karl Thomas, Arch. f. (Anat. u.) Physiol., 1909 and 1910, Suppl. Bd.

can be kept alive for any length of time by food entirely free from fat and carbohydrates.[1] But it has been positively demonstrated that they can be kept alive a long time by feeding exclusively with meat freed as much as possible from visible fat (PFLÜGER[2]). Human beings and herbivora, on the contrary, cannot live for any length of time on such food. On the one hand they lose the property of digesting and assimilating the necessarily large amounts of meat, and on the other a distaste for large quantities of meat or proteins soon appears. The elimination of acetone bodies with an exclusion of carbohydrates from the food of man is of interest (see Chapter XIV).

A question of greater importance is whether it is possible to maintain life in an animal for any length of time with a mixture of simple organic and inorganic foodstuffs. The earlier experiments carried out by many investigators to decide this question have not yielded satisfactory results. and RÖHMANN[3] was first able, by feeding a mixture of several proteins with fat, starch, glucose and salts, to keep mice alive for a long time, and was also able to raise young mice by artificial feeding of the mother and then the small animals. RÖHMANN concludes from his experiments that for the continuous maintenance or for development of the animal a mixture of different proteins is necessary, but more recently he[4] has found that this can be accomplished by a single protein, and the results of his experiment coincide well in this regard with the investigations of OSBORNE and MENDEL (and E. FERRY[5]).

In experiments with white mice these investigators have found that on feeding with a mixture of only one protein with cane-sugar, starch, fat, agar-agar and mineral substances, adult mice could be kept for 169-259 days without changing their body weight. The reason why the adult mice could not be maintained for a still longer time and why young mice did not grow was that certain substances of unknown kind were lacking. Such substances occur in milk, and by adding to the food, milk from which the proteins have been removed, although the food contained only one protein, the animals can be kept alive for a longer time—500–600 days, and the normal growth accomplished as well. These proteins were, especially, casein, lactalbumin, ovalbumin, hemp-seed edestin, wheat glutenin and excelsin, while on the contrary they were not able to pro-

[1] See Horbaczewski, Maly's Jahresber., 31, 715.

[2] Pflüger's Arch., 50.

[3] F. Röhmann, Klin. therap. Wochenschr., No. 40, 1902, and Allg. med. Centralbl. Zeitung, 1908, No. 9.

[4] Röhmann, Bioch. Zeitschr., 39.

[5] Th. B. Osborne and L. B. Mendell, Science, 34. The Carnegie Institution, Washington, Parts 1 and 2, 1911; with Edna Ferry, Journ. of biol. Chem., 12 and 13, and Zeitschr. f. physiol. Chem., 80.

duce a sufficient growth with pea-legumin, zein, gliadin and hordein when added to the other foodstuffs and protein-free milk. These experiments showed that animals fed with gliadin as the only protein had the normal ability to produce offspring and had the ability to produce milk necessary for their food.

In another series of experiments it was shown that the protein-free milk could be replaced by a proper mixture of salts and that the organic constituents of such milk were not necessary. On feeding with fat, carbohydrates, casein and such a salt mixture they were able to attain normal growth in a series of experiments of more than 80 or 100 days. Growth was produced in the animals also in the absence of substances soluble in ether (lipoids). This is remarkable, as according to the observations and experiments of STEPP, lipoids are necessary for the normal nutrition.

According to STEPP [1] a food which is adequate but not quite genuine for mice can be made genuine by the addition thereto of certain substances soluble in alcohol-ether from milk, egg-yolk, brain, etc. These substances, which are neither fat nor cholesterin, and which he calls lipoids, are partly heat-labile and correspondingly lose their action by continuously boiling with alcohol or by a lengthy boiling of the natural food-stuffs with alcohol or water. A proper food for mice can be so changed by continuous boiling with alcohol so that all animals fed with it die, while the changes in the food brought about in this way can be counteracted by the lipoids obtained under conditions where the lengthy action of heat is prevented. Mice, which die with an otherwise sufficient food but free from lipoids may be kept alive by the addition of the undestroyed lipoids to the same food.

Recently it has been suggested that beside the foodstuffs in the ordinary sense, other constituents of our food exist which are of the very greatest importance for life. The investigations of FUNK as well as those of SUZUKI, SHIMAMURA and ODAKE on the constituents of rice-bran give a specially striking proof of this. According to C. FUNK [2] rice-bran contains a substance called *vitamine*, $C_{17}H_{20}N_2O_7$, which belongs to the pyrimidine group and which also occurs in yeast, milk residue and beef-brains. This substance, which is absent in polished rice, causes the disease Beri-Beri in man and polyneuritis in birds. SUZUKI, SHIMAMURA and ODAKE have also isolated from rice-bran a substance which they call *oryzanine*, which is soluble in alcohol and necessary for animal life. With mixtures of protein, carbohydrates, fat and salts without oryzanine these investigators could not keep hens, pigeons and mice alive and dogs could not be

[1] Bioch. Zeitschr., **22**, and Zeitschr. f. Biol., **57** and **59**.

[2] C. Funk, Journ. of Physiol., **43** and **45**; Suzuki, Shimamura and Odake, Bioch. Zeitschr., **43**.

kept alive with boiled meat and polished rice. They emaciate quickly and rapidly recover again if they receive oryzanine.

It follows from the above that there exists a certain unexplainable contradiction between the important observations of STEPP and those of the other investigators on the one side and the very interesting, prolonged experiments of OSBORNE and MENDEL with pure foodstuffs on the other side.

III. METABOLISM WITH VARIOUS FOODS.

For carnivora, as above stated, meat as poor as possible in fat may be a complete and sufficient food. As the proteins moreover take a special place among the organic nutritive bodies by the quantity of nitrogen they contain, it is proper that we first describe the metabolism with an exclusively meat diet.

Metabolism with food rich in proteins, i.e., feeding only with meat as poor in fat as possible.

By an increased supply of proteins the catabolism and the elimination of nitrogen is increased, and this in proportion to the supply of proteins.

If a certain quantity of meat has daily been given to carnivora as food and the quantity is suddenly increased, an augmented catabolism of proteins, or an increase in the quantity of nitrogen eliminated, is the result. If the animal is daily fed for a certain time with larger quantities of the same meat, a part of the proteins accumulates in the body, but this part decreases from day to day, while there is a corresponding daily increase in the elimination of nitrogen. In this way a nitrogenous equilibrium is established; that is, the total quantity of nitrogen eliminated is equal to the quantity of nitrogen in the absorbed proteins or meat. If, on the contrary, an animal in nitrogenous equilibrium, having been fed on large quantities of meat, is suddenly given a small quantity of meat per day, it uses up its own body proteins, the amount decreasing from day to day. The elimination of nitrogen and the catabolism of proteins decrease constantly, and the animal may in this case also pass into nitrogenous equilibrium, or almost into this condition These relations are illustrated by the following table (VOIT):[1]

	Grams of Meat in the Food per Day.	
	Before the Test.	During the Test.
1	500	1500
2	1500	1000

Grams of Flesh Metabolised in Body per Day.

1	2	3	4	5	6	7
1222	1310	1390	1410	1440	1450	1500
1153	1086	1088	1080	1027		

[1] Hermann's Handbuch, 6, Part I, 110.

In the first case (1) the metabolism of meat before the beginning of the actual experiment on feeding with 500 grams of meat was 447 grams, and it increased considerably on the first day of the experiment, after feeding with 1500 grams of meat. In the second case (2), in which the animal was previously in nitrogenous equilibrium with 1500 grams of meat, the metabolism of flesh on the first day of the experiment, with only 1000 grams meat, decreased considerably, and on the fifth day an almost nitrogenous equilibrium was obtained. During this time the animal gave up daily some of its own proteins. Between that point below which the animal loses from its own weight and the maximum, which seems to be dependent upon the digestive and assimilative capacity of the intestinal canal, a carnivore may be kept in nitrogenous equilibrium with varying quantities of proteins in the food.

The supply of proteins, as well as the protein condition of the body, affects the extent of the protein metabolism. A body which has become rich in proteins by a previous abundant meat diet must, to prevent a loss of proteins, take up more protein with the food than a body poor in proteins.

In regard to the rapidity with which the protein catabolism takes place FALTA [1] found in man but not, or at least not to the same extent, in dogs, that quite great differences exist between the different proteins. Thus on feeding pure proteins the chief amount of the nitrogen is more quickly eliminated after feeding casein than after genuine ovalbumin. This latter is more easily demolished after a previous modification by coagulation than in the native state, which indicates that an unequal resistance of the different proteins toward the digestive juices plays a part. HÄMÄLÄINEN and HELME [2] have also obtained similar results. Even on feeding with easily decomposable proteins it always takes several days before the total nitrogen corresponding thereto is eliminated, which depends, according to FALTA, upon a progressive demolition of the protein. From the unequal rate at which the different proteins are decomposed it follows that in the passage from a diet poor in protein to one rich in protein the time within which nitrogenous equilibrium occurs depends chiefly upon the kind of protein contained in the food.

PETTENKOFER and VOIT have made investigations on the *metabolism of fat* with an exclusively protein diet. These investigations have shown that by increasing the quantity of proteins in the food the daily metabolism of fat decreases, and they have drawn the conclusion from these experiments, that there may even take place a formation of fat under these circumstances. The objections presented by PFLÜGER to these

[1] Deutsch. Arch. f. klin. Med., 86.
[2] Skand. Arch. f. Physiol., 19.

experiments, as well as the proofs of the formation of fat from proteins, are also given in Chapter IX.

According to PFLÜGER'S doctrine, the protein can influence the formation of fat only in an indirect way, namely, in that it is consumed instead of the non-nitrogenous bodies and hence the fat and fat-forming carbohydrates are spared. If sufficient protein is introduced with the food to satisfy the total nutritive requirements, then the decomposition of fat stops; and if non-nitrogenous food is taken at the same time, this is not consumed, but is stored up in the animal body, the fats as such, and the carbohydrates at least in great part as fat.

PFLÜGER defines the "nutritive requirement" as the smallest quantity of lean meat which produces nitrogenous equilibrium without causing any decomposition of fat or carbohydrates. At rest and at an average temperature it is found in dogs to be 2.073 to 2.099 grams of nitrogen [1] (in meat fed) per kilo of flesh weight (not body weight, as the fat, which often forms a considerable fraction of the weight of the body, cannot as it were be used as dead measure). Even when the supply of protein is in excess of the nutritive requirements, PFLÜGER found that the protein metabolism increases with an increased supply until the limit of digestive power is reached, which limit is about 2600 grams of meat with a dog weighing 30 kilos. In these experiments of PFLÜGER'S not all of the excess of protein introduced was completely decomposed, but a part was retained by the body. PFLÜGER therefore defends the proposition "that a supply of proteins only, without fat or carbohydrate does not exclude a protein fattening."

From what has been said on protein metabolism in starvation and with exclusive protein food, it follows that the protein catabolism in the animal body never stops, that the extent is dependent in the first place upon the extent of protein supply, and that the animal body has the property, within wide limits, of accommodating the protein catabolism to the protein supply.

These and certain other peculiarities of protein catabolism have led VOIT to the view that not all proteins in the body are decomposed with the same ease. VOIT differentiates between the proteins fixed in the tissue-elements, so-called organized proteins, *tissue-proteins*, from those proteins which circulate with the fluids in the body and its tissues and which are taken up by the living cells of the tissues, from the interstitial fluids washing them, and destroyed. These *circulating proteins* or *supply proteins* are, he claims, more easily and quickly destroyed than the tissue-proteins. When, therefore, in a fasting animal which has been previously fed with meat, an abundant and quickly decreasing decomposition of proteins takes place, while in the further course of starvation this protein catabolism becomes less in quantity and more uniform, this depends upon the fact that the supply of circulating proteins is destroyed chiefly in the first days of starvation and the tissue-proteins in the last days.

The tissue-elements constitute an apparatus of a relatively stable nature, which has the power of taking proteins from the fluids washing the tissues and appropriating them, while their own proteins, the tissue-

[1] See Schöndorff, Pflüger's Arch., 71.

proteins, are ordinarily catabolized to only a small extent, about 1 per cent daily (VOIT). By an increased supply of proteins the activity of the cells and their ability to decompose nutritive proteins is also increased to a certain degree. When nitrogenous equilibrium is obtained after an increased supply of proteins, it indicates that the decomposing power of the cells for proteins has increased so that the same quantity of proteins is metabolized as is supplied to the body. If the protein metabolism is decreased by the simultaneous administration of other non-nitrogenous foods (see below), a part of the circulating proteins may have time to become fixed and organized by the tissues, and in this way the mass of the flesh of the body increases. During starvation or with a lack of proteins in the food the reverse takes place, for a part of the tissue proteins is converted into circulating proteins which are metabolized, and in this case the flesh of the body decreases.

VOIT'S theory has been criticised by several investigators and especially by PFLÜGER. PFLÜGER'S belief, based on an investigation made by one of his pupils, SCHÖNDORFF,[1] is that the extent of protein destruction is not dependent upon the quantity of circulating proteins, but upon the nutritive condition of the cells for the time being—a view which does not widely differ from VOIT if the author does not misunderstand PFLÜGER. VOIT[2] has, as is known, stated that the conditions for the destruction of substances in the body exist in the cells, and also that the circulating protein is first catabolized after having been taken up by the cells from the fluids washing them. Besides this, certain investigations conclusively show that the extent of protein catabolism is dependent upon the concentration of the decomposable proteins at the place where the decomposition is taking place. Thus in confirmation with the earlier investigations of v. GEBHARDT and KRUMMACHER, THOMAS, v. HOESSLIN and LESSER[3] have recently shown that on feeding with a certain quantity of protein, less protein was catabolized when the protein was supplied piecemeal, i.e., in several small portions during the day instead of at one time. That the peculiarity of the nitrogen elimination in starvation and after sufficient protein supply depends essentially upon the concentration of the decomposable proteins (or more correctly the decomposable nitrogenous substances) is no doubt also generally admitted.[4]

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[1] Pflüger, Pflüger's Arch., **54**; Schöndorff, *ibid.*, **54**.

[2] Zeitschr. f. Biologie, **11**.

[3] K. Thomas. Arch. f. (Anat. u.) Physiol., 1909; H. v. Hoesslin and E. J. Lesser, Zeitschr. f. physiol. Chem., **73**, when also the works of v. Gebhardt and Kummacher are cited.

[4] See also E. Voit and A. Korkunoff, Zeitschr. f. Biol., **32**, and O. Frank and R. Trommsdorff, *ibid.*, **43**.

Recent investigations, especially those of FOLIN,[1] which show that the amount of certain nitrogenous urinary constituents, such as creatinine, uric acid and the combinations containing neutral sulphur, are almost independent of the quantity of protein taken as food, while the quantity of urea is determined by the protein partaken of, tend to substantiate VOIT'S view that we must differentiate between the real cell protein and the food protein. This has also led FOLIN to differentiate between endogenous and exogenous protein metabolism. The chief point in VOIT'S theory that all the proteins in the body do not behave alike and that the organized proteins which have been fixed in the cells and have been introduced into the cell structure are less readily catabolized than the proteins occurring in the nutritive fluids or temporarily taken up from these, must also be considered as not disputed. RUBNER[2] differentiates also between the deposited protein (growth protein, and deposited by the activity of the cells melioration protein) in the body on the one hand and the protein temporarily incorporated with the body (supply protein and catabolized in passing to a protein-poor diet, transitory protein) on the other hand.

This question is intimately connected with another, namely, whether the food proteins taken up by the cells are metabolized as such or whether they are first organized, i.e., are converted into specific cell protein. The observations of PANUM, FALCK, ASHER and HAAS and others[3] on dogs have shown that the nitrogen elimination increases almost immediately after a meal and in the fifth or sixth hour according to these experimenters, when according to SCHMIDT-MULHEIM[4] about 59 per cent of the eaten protein is absorbed, do not indicate that a transformation of the food protein into organized protein occurs before it is catabolized. The recent investigations upon the deep cleavage of proteins in digestion and the generally accepted protein syntheses from amino-acids have made this question lose its special interest.

On account of the above-stated action of the concentration of the catabolizable nitrogenous material upon the protein decomposition or nitrogen elimination, it is not possible to replace the quantity of protein catabolized in starvation by the exclusive feeding of protein administered at one time and in quantities corresponding to the food proteins. This always requires large amounts of protein. Even on the fractional introduction of natural protein v. HOESSLIN and LESSER were unable to produce a nitrogen equilibrium with quantities of protein equal to the starva-

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[1] Amer. Journ. of Physiol., **13**.

[2] Arch. f. (Anat. u.) Physiol., 1911.

[3] Panum, Nord. Med. Arkiv., **6**; Falck, see Hermann's Handbuch, **6**, Part I, 107; Asher and Haas, Bioch. Zeitschr., **12**. For further information in regard to the curve of nitrogen elimination in man, see Tschenloff, Korrespond. Blatt Schweiz. Aerzte, 1896; Rosemann, Pflüger's Arch., **65**, and Veraguth, Journ. of Physiol., **21**; Schlosse, Maly's Jahresber., **31**.

[4] Arch. f. (Anat. u.) Physiol., 1879.

tion protein; the elimination of nitrogen was always somewhat increased. On the fractional introduction of protein, THOMAS[1] was nevertheless able in dogs to produce nitrogenous equilibrium without essentially raising the protein metabolism (in comparison with the starvation value). In experiments upon himself he was not able to produce this.

It has been stated above that other foods may decrease the catabolism of proteins. Gelatin is such a food. *Gelatin* and the *gelatin-formers* do not seem to be converted into protein in the body, and this last cannot be entirely replaced by gelatin in the food. For example, if a dog is fed on gelatin and fat, its body sustains a loss of proteins even when the quantity of gelatin is great enough so that the animal with an amount of fat and meat containing just the same quantity of nitrogen as the gelatin in question, remains in nitrogenous equilibrium. On the other hand, gelatin, as VOIT, PANUM and OERUM[2] have shown, has great value as a means of sparing the proteins, and it may decrease the catabolism of proteins to a still greater extent than fats and carbohydrates. This is apparent from the following summary of VOIT's experiments upon a dog:

Food per Day.				Flesh.	
Meat.	Gelatin.	Fat.	Sugar.	Catabolised.	On the Body.
400	0	200	0	450	−50
400	0	0	250	439	−39
400	200	0	0	356	+44

I. MUNK[3] has later arrived at similar results by means of more decisive experiments, and the recent investigations of KRUMMACHER and KIRCHMANN[4] show the extent of the sparing action of gelatin upon proteins. The extent of protein destruction during gelatin feeding was compared with the extent of protein catabolism in starvation, and it was found that 35–37.5 per cent of the quantity of protein decomposed in starvation could be spared by gelatin. The physiological availability of gelatin was found by KRUMMACHER to be equal to 3.88 calories for 1 gram, which corresponds to about 72.4 per cent of the energy-content of the gelatin.

The value of gelatin has been found by MURLIN[5] to be dependent to a high degree upon the protein condition of the body, on the calorific value of the food and the quantity of carbohydrates in the latter. If in a man weighing 70 kilos, 51 calories per kilo were partaken, the quantity of nitrogen eliminated was 10 per cent more than the starvation

[1] v. Hoesslin and Lesser, l. c.; Thomas, Arch. f. (Anat. u.) Physiol., Suppl. Bd., 1910.
[2] Voit, l. c., 123; Panum and Oerum, Nord. Med. Arkiv., **11**.
[3] Pflüger's Arch., **58**.
[4] Krummacher, Zeitschr. f. Biologie, **42**; Kirchmann, *ibid.*, **40**.
[5] Amer. Journ. of Physiol., **19**.

value, and when two-thirds of the total calories partaken of were supplied by carbohydrates, 63 per cent of the total nitrogen could be replaced by gelatin nitrogen.

The reason why gelatin cannot entirely replace protein has been sought for in the fact that gelatin does not contain all the amino-acids of the proteins (such as tyrosine and tryptophane), or does not contain a sufficient amount of the various amino-acids. The correctness of this explanation was first shown by KAUFMANN by an experiment on himself, where he showed that gelatin after addition of tyrosine, tryptophane and cystine could be made equivalent to protein. The conclusive proof was given later by ABDERHALDEN [1] when he showed that completely decomposed gelatin on the addition of a mixture of amino-acids, among them also tyrosine and tryptophane, could be made equivalent to proteins.

As it has been possible to replace the proteins in the food by their cleavage products or mixtures of amino-acids,[2] it is easily understandable that also proteoses or peptones can completely or partly replace the protein. Their ability in this regard is essentially dependent upon their constitution, i.e., their content of the different amino-acids. As the proteoses and peptones are produced by cleavage and as therefore in one proteose we hae certain atomic comp lexes and in others again these may be absent or only exist to a slight extent, it is conceivable that different investigators [3] have obtained contradictory results because of the use of different proteoses and peptones.

We have a number of investigations on the action of amides upon metabolism, which are mostly connected by the use of *asparagin*. These investigations have in part led to conflicting results; but they indicate that carnivora and herbivora act differently, that the results are dependent upon the rapidity with which the asparagin is absorbed and also upon the bacterial action in the intestine, and that in herbivora a protein-sparing action can be brought about by asparagin.[4] If, as is generally

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[1] Martin Kaufmann, Pflüger's Arch., **109**; Abderhalden, Zeitschr. f. physiol. Chem., **77**.

[2] See Abderhalden and collaborators, Chapter VIII; also Abderhalden, Zeitschr. f. physiol. Chem., **77**, and especially **83**.

[3] In regard to the literature on the nutritive value of the proteoses and peptones see Maly, Pflüger's Arch., **9**; Plósz and Gyergyay, *ibid.*, **10**; Adamkiewicz, 'Die Natur und der Nährwerth des Peptones" (Berlin, 1877); Pollitzer, Pflüger's Arch., **37**, 301; Zuntz, *ibid.*, **37**, 313; Munk, Centralbl. f. d. med. Wissensch., 1889, 20, and Deutsch. med. Wochenschr., 1889; Ellinger, Zeitschr. f. Biologie, **33** (literature). Blum, Zeitschr. f. physiol. Chem., **30**; Henriques and Hansen, Zeitschr. f. physiol. Chem., **48**.

[4] Weiske, Zeitschr. f. Biologie, **15** and **17**, and Centralbl. f. d. med. Wissensch., 1890, 945; Munk, Virchow's Arch., **94** and **98**; Politis, Zeitschr. f. Biologie, **28**. See also Mauthner, *ibid.*, **28**; Gabriel, *ibid.*, **29**; and Voit, *ibid.*, **29**, 125; Kellner, Maly's Jahres-

admitted, the amino-acids can serve in the building up of the proteins, then there is no use denying that their amides can also be used by the animal body.

Recently GRAFE, ABDERHALDEN[1] and their collaborators have carried on investigations on the value of ammonia and of urea as protein sparers and protein formers. These investigations have shown that ammonia or urea under special conditions of experimentation may cause a nitrogen retention, but we are not justified in believing that a synthesis of protein from ammonia takes place.

Metabolism on a Diet Consisting of Protein, with Fat or Carbohydrates. As the various foodstuffs can replace each other as sources of energy in the food it follows that the non-nitrogenous foodstuffs can be used instead of the proteins and can reduce the catabolism of these. Thus the fat cannot completely arrest or prevent the catabolism of proteins, but it can decrease it and so spare the proteins. This is apparent from the following table by VOIT.[2] A is the average for three days, and B for six days.

	Food.		Flesh.	
	Meat.	Fat.	Metabolized.	On the Body.
A.....	1500	0	1512	−12
B.....	1500	150	1474	+26

According to VOIT the adipose tissue of the body acts like the food-fat, and the protein-sparing effect of the former may be added to that of the latter, so that a body rich in fat may not only remain in nitrogenous equilibrium, but may even add to the store of body proteins, while in a lean body with the same food containing the same amount of proteins and fat there would be a loss of proteins. In a body rich in fat a greater quantity of proteins is protected from metabolism by a certain quantity of fat than in a lean body.

Like the fats the carbohydrates have a sparing action on the proteins. By the addition of carbohydrates to the food, carnivora not only remain in nitrogenous equilibrium, but the same quantity of meat which in itself is insufficient and which without carbohydrates would cause a loss

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ber., **27**, and Zeitschr. f. Biologie, **39**; Pflüger's Arch., **113**; Kellner and Köhler, Chem. Centralbl., **1**, 1906; Völtz, Pflüger's Arch., **107**, **117**, with Yakuwa, *ibid.*, **121**; v. Strusiewicz, Zeitschr. f. Biol., **47**; Rosenfeld and Lehmann, Pflüger's Arch., **112**; Lehmann, *ibid.*, **115**; M. Müller, *ibid.*, **117**; Henriques and Hansen, Zeitschr. f. physiol. Chem., **54**.

[1] Grafe, Zeitschr. f. physiol. Chem., **78**, **82**, **84**, with Schläpfer, *ibid.*, **77**, with Turban, *ibid.*, **83**; Völtz, *ibid.*, **74**; Abderhalden with Hirsch or Laupé, *ibid.*, **80**, **82-84**; Peschek, Bioch. Zeitschr. **45**.

[2] Voit, in Hermann's Handb., **6**, 130.

of weight in the body may with the addition of carbohydrates produce a deposit of proteins. This is apparent from the following table:[1]

Food.				Flesh.	
Meat.	Fat.	Sugar.	Starch.	Metabolised.	On the Body.
500	250	...	...	558	− 58
500	...	300	...	466	+ 34
500	...	200	...	505	− 5
800	...	...	250	745	+ 55
800	200	...	...	773	+ 27
2000	...	...	200–300	1792	+208
2000	250	...	...	1883	+117

The sparing of protein by carbohydrates is greater, as shown by the table, than by fats. According to VOIT the first is on an average 9 per cent and the other 7 per cent of the administration protein without a previous addition of non-nitrogenous bodies. Increasing quantities of carbohydrates in the food decrease the protein metabolism more regularly and constantly than increasing quantities of fat. ATWATER and BENEDICT[2] also found that the carbohydrates had a somewhat greater sparing action upon proteins than fats.

Because of this great protein-sparing action of carbohydrates the herbivora, which as a rule partake of considerable quantities of carbohydrates, assimilate proteins readily (VOIT).

The greater protein-sparing action of carbohydrates as compared with that of the fats occurs, as shown by LANDERGREN,[3] to a still higher degree with food poor in nitrogen or in nitrogen starvation, in which cases the carbohydrates have double the protein-sparing action as compared with an isodynamic quantity of fat. This different behavior of the fats and the carbohydrates is also shown in the experiments of RUBNER and THOMAS[4] that on the exclusive feeding of sugar the nitrogen elimination is reduced to the wear and tear quota while on the exclusive feeding of fats the nitrogen requirement was about two to three times as great as the wear and tear quota.

The protein-sparing action of the carbohydrates and fats has generally been studied through the one-sided feeding with one or the other of these two groups of foodstuffs. The question may be raised whether the difference observed between the fats and carbohydrates could not also be brought about by the simultaneous supply of carbohydrates and fat in varying proportions. TALLQUIST[5] made a series of experiments on this

[1] Voit, in Hermann's Handb., 6, page 143.

[2] See Ergebnisse der Physiologie, 8.

[3] l. c., Inaug.-Diss., and Skand. Arch. f. Physiol., 14. Wimmer, Zeitschr. f. Biol., 57, has given further proofs of the strong protein-sparing action of carbohydrates in nitrogen starvation.

[4] See Thomas, Arch. f. (Anat. u.) Physiol. Suppl. Bd., 1910.

[5] Finska Lakaresällskapets Handl., 1901. See also Arch. f. Hygiene, 41.

subject. In one of the periods 16.27 grams N, 44 grams fat, and 466 grams carbohydrate were given; in a second, 16.08 grams N, 140 grams fat, and 250 grams carbohydrate, containing almost the same number of calories, namely, 2867 and 2873. In both cases an almost complete nitrogenous equilibrium was reached and the carbohydrate did not spare more protein than the fat. It is therefore possible that the fat has about the same protein-sparing action as an isodynamic amount of carbohydrate when the quantity of carbohydrates does not sink below a certain minimum, which is not known for the present.

This condition as well as the extent of protein-sparing action of the carbohydrates stands, according to LANDERGREN, in close relation to the formation of sugar in the body. The animal body always needs sugar, and a lack of carbohydrates in the food leads to a part of the proteins being used in the sugar formation. This part can be spared by carbohydrates but not by fats, from which, according to LANDERGREN, the carbohydrates cannot be formed. In this also lies the probable reason why the fats, on being fed exclusively but not with a sufficient supply of carbohydrates, have a much lower protein-sparing action than the carbohydrates. The fats cannot prevent the protein catabolism necessary for the formation of sugar on a diet lacking in carbohydrates.

The law as to the increased protein catabolism with increased protein supply also applies to food consisting of protein with fat and carbohydrates. In these cases the body tries to adapt its protein catabolism to the supply; and when the daily calorie-supply is completely covered by the food, the organism can, within wide limits, be in nitrogenous equilibrium with different quantities of protein.

The upper limit to the possible protein catabolism per kilo and per day has been determined only for herbivora. For human beings it is not known, and its determination is from a practical standpoint of secondary importance. What is more important is to ascertain the lower limit, and on this subject we have several older experiments upon man as well as upon dogs by HIRSCHFELD, KUMAGAWA, KLEMPERER, MUNK, ROSENHEIM,[1] and others. It follows from these experiments that the lower limit of protein requirement for human beings, for a week or less, is about 30–40 grams or 0.4–0.6 gram per kilo with a body of average weight. v. NOORDEN[2] considers 0.6 gram protein (absorbed protein) per kilo and per day as the lower limit (threshold of protein requirement). The above-mentioned figures are valid only for short series of experiments; still there exists the observation of E. VOIT and CONSTANTINIDI[3] on

[1] See footnote 3, page 903; also Munk, Arch. f. (Anat. u.) Physiol., 1891 and 1896 Rosenheim, *ibid.*, 1891; Pflüger's Arch., 54.

[2] Grundriss einer Methodik der Stoffwechseluntersuchungen. Berlin, 1892.

[3] Zeitschr. f. Biologie, 25.

the diet of a vegetarian when the protein condition was kept almost but not completely normal for a long time with about 0.6 gram of protein per kilo. CASPARI [1] has also made observations upon a vegetarian for a period of 14 days with an average of 0.1 gram nitrogen (recalculated as equal to 0.62 gram protein) per kilo, where a nearly complete nitrogenous equilibrium was observed as the average result.

According to VOIT's normal figures, which will be spoken of below, for the nutritive need of man, an average workingman of about 70 kilos weight, requires on a mixed diet about 40 calories per kilo (true calories or net calories). In the above experiments with food very poor in protein the demand for calories was considerably greater; as, for instance, in certain cases it was 51 (KUMAGAWA) or even 78.5 calories (KLEMPERER). It therefore seems as if the above very low supply of protein was possible only with great waste of non-nitrogenous food; but in opposition to this it must be recalled that in VOIT and CONSTANTINIDI'S experiments upon the vegetarian, who for years was accustomed to a food poor in protein and rich in carbohydrate, the calories amounted to only 43.7 per kilo. In the case studied by CASPARI a supply of 41 calories per kilo was entirely sufficient.

SIVÉN has shown by experiments upon himself that the adult human organism, at least for a short time, can be maintained in nitrogenous equilibrium with a specially low supply of nitrogen without increasing the calories in the food above the normal. With a supply of 41–43 calories per kilo he remained in nitrogenous equilibrium for four days with a supply of nitrogen of 0.08 gram per kilo of body weight. Of the nitrogen taken, a part was of a non-protein nature and the quantity of true protein nitrogen was only 0.045 gram, corresponding to about 0.3 gram of protein per kilo of body weight. That this low limit, which by the way holds only for a short time, has no general validity follows from other observations. Thus CASPARI [2] also, in an experiment on himself, could not attain complete nitrogenous equilibrium on a much greater nitrogen supply. The protein minimum also seems to vary in different individuals.

The protein minimum can also be different for other reasons. It varies, as mentioned by RUBNER, not only with the kind of foodstuffs, but also with the nutritive condition of the body. The needs of the cells for protein varies with the nutritive condition of the body. Where the protein is eagerly demanded, less supply of protein suffices, and where the demand is low more protein must be offered (RUBNER). The more the

[1] Physiologische Studien über Vegetarismus, Bonn, 1905.

[2] Sivén, Skand. Arch. f. Physiol., 10 and 11; Caspari, Arch. f. (Anat. u.) Physiol., 1901.

body has become reduced the lower is the protein minimum, according to RUBNER.[1]

As mentioned in the early part of this chapter, the body always suffers a certain loss of nitrogen through the falling out of the hair and other epidermis formations, by the secretions, etc.; but to this also belongs the constant loss of nitrogenous substance which every cell sustains because of its activity. This unpreventable loss of nitrogen has been included by RUBNER under the name "*wear and tear*" *quota*, and this quota, which corresponds to the nitrogen elimination with a perfectly nitrogen-free diet, and hence is a protein minimum, may rise to 4 to 6 per cent of the total calorific needs. The energy supply of the food is under these conditions entirely assumed by the non-nitrogenous foodstuffs, and when this quota is replaced by protein the body is in a condition of lowest nitrogenous equilibrium.

All proteins do not have the same value in replacing the protein minimum. MICHAUD[2] determined the protein minimum in dogs by feeding entirely with nitrogen-free food, and he found that this minimum can be covered by the corresponding quantity of protein specific of the animal, but not by the same quantity of an alien protein, like gliadin and edestin. v. HOESSLIN and LESSER have found on the contrary in experiments with dogs that proteins specific to the animal were only unessentially superior to the proteins of horse flesh, and E. VOIT and LISTERER found for the three kinds of protein, beef-muscle, aleuronat and casein, that the relation was 100 : 106 : 121. THOMAS[3] has carried out experiments on man with different foods and has found that the nitrogen of various kinds of proteins has an unequal value in replacing the wear and tear quota. By the expression "*biological equivalence*" of the nitrogenous foodstuffs he denotes the number of parts of body nitrogen which can be replaced by 100 parts of the food-nitrogen and he found the following equivalence: for beef = 104.7, milk = 99.7, casein = 70.14, wheat flour = 39.6, potatoes = 78.9, peas = 55.7, and corn = 29.5. Also in consideration of the different content of nitrogenous extractives in the food these figures therefore show that different proteins have essentially different values for the replacement of the nitrogen minimum.

The purposes of the protein as foodstuff are, according to RUBNER, as follows: (1) To compensate for the wear and tear quota; (2) betterment of the condition of the cells; and (3) dynamogenic purpose. In the accomplishment of this third purpose the protein splits into a nitrogenous

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[1] Rubner, Theorie d. Ernährung nach Vollendung des Wachstums, Arch. f. Hyg., **66**, 1–80, and Ernährungsorgänge beim Wachstum des Kindes, *ibid.*, **66**, 81–126.

[2] Zeitschr. f. physiol. Chem., **59**.

[3] v. Hoesslin and Lesser, l. c., E. Voit and Listerer, Zeitschr. f. Biol., **53**; Thomas, Arch. f. (Anat. u.) Physiol., 1909.

and a non-nitrogenous part. The potential energy set free immediately as heat in the combustion of the nitrogenous part, which is quantitatively used within the region of the chemical heat regulation but is otherwise lost, has been called the *specific dynamic action* by RUBNER.[1] The remainder of the energy which is represented by the non-nitrogenous part of the proteins, serves, like all other foodstuffs, in satisfying the energy requirement of the cells. According to RUBNER only non-nitrogenous groups (of the proteins, fats and carbohydrates) come almost entirely, if not completely, in consideration for purposes of energy.

In close relation to the second purpose, the betterment of the condition of the cells, stands the question as to the conditions favoring the deposition of flesh in the body, which is closely associated with the question as to the conditions of fattening the body. In this connection it must be remembered in the first place that all fattening presupposes an overfeeding, i.e., a supply of foodstuffs which is greater than that catabolized in the same time.

In carnivora a flesh deposition may take place on the exclusive feeding with meat. This is not generally large in proportion to the quantity of protein catabolized. In man and herbivora, who cannot cover their calorific needs by protein alone, this is not possible, and the question as to the conditions of fattening with a mixed diet is of importance.

These conditions have also been studied in carnivora, and here, as VOIT has shown, the relation between protein and fat (and carbohydrates) is of great importance. If much fat is given in proportion to the protein of the food, as with average quantities of meat with considerable addition of fat, then nitrogenous equilibrium is but slowly attained and the daily deposit of flesh, though not large, is quite constant, and may become greater in the course of time. If, on the contrary, much meat besides proportionately little fat is given, then the deposit of protein with increased catabolism is smaller day by day, and nitrogenous equilibrium is attained in a few days. In spite of the somewhat larger deposit *per diem*, the total flesh deposit is not considerable in these cases. The following experiment of VOIT may serve as example:

Number of Days of Experimentation.	Food.		Total Deposit of Flesh.	Daily Deposit of Flesh.	Nitrogenous Equilibrium.
	Meat, Grams.	Fat, Grams.			
32	500	250	1792	56	Not attained
7	1800	250	854	122	Attained

The greatest absolute deposition of flesh in the body was obtained in these cases with only 500 grams of meat and 250 grams of fat, and even

[1] Rubner, l. c., and Gesetze des Energieverbrauches, 70.

after 32 days nitrogenous equilibrium had not occurred. On feeding with 1800 grams of meat and 250 grams of fat nitrogenous equilibrium was established after seven days; and though the deposition of flesh per day was greater, still the absolute deposit was not one-half as great as in the former case.

The possibility of a protein fattening in man and animals (dogs, sheep) is shown by the series of experiments of KRUG, BORNSTEIN, SCHREUER, HENNEBERG, PFEIFFER and KALB and others[1] and there is no doubt that such a fattening is possible. That we are here not dealing with an increase in the number of cells, but rather an enlargement of the volume of the same is the generally accepted view. Theories as to the value and nature of this protein-fattening are still divergent, as we must differentiate between flesh accumulation or actual organ formation and protein accumulation or deposition of dead protein, and opinions vary in regard to the question how far the one or the other of these occur. By determining the relation between P_2O_5 and N in muscles, kidneys and liver in dogs and hens in starvation and in fattening, GRUND[2] has tested this possibility experimentally. If we are dealing with the deposition of dead protein then the relationship of the P_2O_5 to the N would change in favor of the nitrogen; GRUND found only a very slight change of this kind, which was not conclusive, and according to him the various organs have correspondingly a certain tendency of maintaining the relation between phosphorus and nitrogen unchanged in starvation as well as in fattening.

It is difficult to produce a permanent flesh deposit in adult man by overfeeding alone. It is to a much greater degree a function of the specific growth energy of the cells and the cell-work than the excess of food. Therefore there is observed, according to v. NOORDEN, abundant flesh deposition (1) in each growing body; (2) in those no longer growing, but whose body is accustomed to increased work; (3) whenever, by previous insufficient food or by disease, the flesh condition of the body has been diminished and therefore requires abundant food to replace it. The deposition of flesh is in this case an expression of the regenerative energy of the cells.[3]

The experiences of graziers show that in food-animals a flesh deposit does not occur, or at least is only inconsiderable, on overfeeding. The

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[1] Krug, Cited by v. Noorden, Lehrb. der Path. des Stoffwechsel, 1. Aufl., p. 120; Bornstein, Berl. klin. Wochenschr., 1898, and Pflüger's Arch., **83** and **106**; Bornstein and Schreuer, Pflüger's Arch., **110**; Henneberg and Pfeiffer, see Maly's Jahresb., **20**; Pfeiffer and Kalb, *ibid.*, **22**.

[2] G. Grund, Zeitschr. f. Biol., **54**.

[3] See also Svenson, Zeitschr. f. klin. Med., **43**.

individuality and the race of the animal are of importance for flesh deposition.

The conditions in young, growing individuals differ from those in adults. In the first the protein is necessary for the building up of the growing tissue and in them an abundant true flesh deposition takes place. For this protein fattening the amount of supply does not take first place, but rather the energy of development.

As above stated (Chapter IX), in regard to the formation of fat in the animal body, the most essential condition for a fat deposition is an overfeeding with non-nitrogenous foods. The extent of fat deposition is determined by the excess of calories administered over those actually needed. But as protein and fat are expensive nutritive bodies as compared with carbohydrates, the supply of greater quantities of carbohydrates is important for fat deposition. The body decomposes less substances at rest than during activity. Bodily rest, besides a proper combination of the three chief groups of organic foods, is therefore also an essential requisite for an abundant fat deposit.

E. GRAFE and D. GRAHAM [1] report an experiment on a dog in which they were able to keep the body weight nearly constant for about two months by excessive food with about 210 per cent of the minimum need of calories and with a diet very rich in non-nitrogenous food-stuffs. No fattening occurred in this case; the calories produced were considerably increased and the author considers this case as an accommodation to the food and a luxus-consumption of non-nitrogenous food-stuffs.

Action of Certain Other Bodies on Metabolism. *Water.* If a quantity in excess of that which is necessary, is introduced into the organism, the excess is quickly and principally eliminated with the urine. This increased elimination of urine causes in fasting animals (VOIT, FORSTER), but not to any appreciable degree in animals taking food (SEEGEN, SALKOWSKI and MUNK, MAYER, DUBELIR [2]), an increased elimination of nitrogen. The reason for this increased nitrogen excretion is to be found in the fact that the drinking of much water causes a complete washing out of the urea from the tissues. Another view, which is defended by VOIT, is that because of the more active current of fluids, after taking large quantities of water, an increased metabolism of proteins takes place. VOIT considers this explanation the correct one, although he does not deny that by the liberal administration of water a more complete washing out of the urea from the tissues takes place. Opinions on this subject are not yet in accord, and recently HEILNER has advocated VOIT'S

[1] Zeitschr. f. physiol. Chem., 73.

[2] Voit, Untersuch. über den Einfluss des Kochsalzes, etc. (München, 1860); Forster, cited from Voit in Hermann's Handbuch, 6, 153; Seegen, Wien. Sitzungsber., 63; Salkowski and Munk, Virchow's Arch., 71; Mayer, Zeitschr. f. klin. Med., 2; Dubelir, Zeitschr. f. Biologie, 28.

view. The recent investigations of ABDERHALDEN [1] show a washing out of the retained nitrogen by the partaking of water.

We have the thorough investigations of HAWK [2] and his co-workers on the action of drinking of water upon the digestion and absorption of foods as well as upon the putrefaction processes in the intestine and the elimination of allantoin and purine bodies in the urine.

When the body has lost a certain amount of water, then the abstinence from water (in animals) is accompanied by a rise in the protein metabolism (LANDAUER, STRAUB [3]). In regard to the action of water on the formation of fat and its metabolism, the theory that the free drinking of water is favorable for the deposition of fat seems to be generally admitted, while the drinking of only very little water acts against its formation. For the present we have no conclusive proofs of the correctness of this view.

Salts. In regard to the action of salts—for example sodium chloride and the neutral salts—which partly depends upon the use of large and varying amounts of salt in the experiments, the authors disagree. Investigations of STRAUB and ROST [4] show that the action of salts stands in close relation to their power of abstracting water. Small amounts of salt which do not produce diuresis have no action on metabolism. On the contrary, larger amounts, which bring about a diuresis, which is not compensated by the ingestion of water, produce a rise in the protein metabolism. If the diuresis is compensated by drinking water, then the protein metabolism is not increased by salts, but is diminished to a slight degree. An increased nitrogen excretion caused by taking salts can be increased by the ingestion of water, thus increasing the diuresis, and the action of salts seems to bear a close relation to the demand and supply of water.

Alcohol. The question as to how far the alcohol absorbed in the intestinal canal is burnt in the body, or whether it leaves the body unchanged by various channels, has been the subject of much discussion. To all appearances the greatest part of the alcohol introduced (95 per cent or more) is burnt in the body (STUBBOTIN, THUDICHUM, BODLÄNDER, BENEDICENTI [5]). As the alcohol has a high calorific value (1 gram = 7.1

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[1] See R. Neumann, Arch. f. Hygiene, **36**; Heilner, Zeitschr. f. Biologie, **47** and **49**; Hawk, University of Pennsylvania Med. Bull., xviii; Abderhalden, Zeitschr. f. physiol. Chem., **59**.

[2] See Journ. of biol. Chem., **10** and **11**, Arch. of internat. Med., 1911, Journ. of Amer. Chem. Soc., **33** and **34**.

[3] Landauer, Maly's Jahresber., **24**; Straub, Zeitschr. f. Biologie, **37**.

[4] W. Straub, Zeitschr. f. Biologie, **37** and **38**; Rost, Arbeiten aus d. Kaiserliche Gesundheitsamte, **18** (literature). See also Grüber, Maly's Jahresber., **30**, 612.

[5] Arch. f. (Anat. u.) Physiol., 1896. which contains the literature.

calories), then the question arises whether it acts sparingly on other bodies, and whether it is to be considered as a nutritive substance. The earlier investigations made to decide these questions have led to no decisive result. The thorough investigations of ATWATER and BENEDICT, ZUNTZ and GEPPERT, BJERRE, CLOPATT, NEUMANN, OFFER, ROSEMANN,[1] and others, seem to show positively that, in man, alcohol can diminish the consumption not only of fat and carbohydrates, but also the proteins, although at first, due to its poisonous properties, it may increase the protein metabolism for a short time. The nutritive value of alcohol can be of special importance in certain cases only, as large amounts of alcohol taken at one time, or the continued use of smaller quantities, has an injurious action on the organism. Alcohol may therefore be regarded as a foodstuff only in exceptional cases, and in other respects must be considered as an article of luxury.

Coffee and *tea* have no action on the exchange of material which can be positively proven, and their importance lies chiefly in their action upon the nervous system. It is impossible to enter into the effect of various therapeutic agents upon metabolism.

IV. THE DEPENDENCE OF METABOLISM ON OTHER CONDITIONS.

The so-called basal requirement which was previously mentioned, i.e., the extent of metabolism with absolute rest of body and inactivity of the intestinal tract, serves best as a starting-point for the study of metabolism under various external circumstances. The metabolism going on under these conditions leads in the first place to the production of heat, and it is only to a subordinate degree dependent upon the work of the circulatory and respiratory apparatus and the activity of the glands. According to a calculation by ZUNTZ,[2] only 10–20 per cent of the total calories of the basal requirement belongs to the circulation and respiration work.

The magnitude of the basal requirement depends in the first place upon the heat production necessary to cover the loss of heat, and this heat production is in turn dependent upon the relation between the weight and the surface of the body.

Weight of Body and Age. The greater the mass of the body the greater the absolute consumption of material; while, on the contrary, other

[1] In regard to the literature on this subject, see the works of O. Neumann, Arch. f. Hygiene, **86** and **41**, and Rosemann, Pflüger's Arch., **86** and **94**. A summary of the entire literature upon alcohol can be found in Abderhalden, "Bibliographie der gesamten wissenschaftlichen Literatur über den Alcohol und den Alcoholismus," Berlin and Wien, 1904. See also Rosemann in Oppenheimer's Handb. d. Bioch., Bd. 4, 1.

[2] Cited from v. Noorden's Handbuch, 1. Aufl., page 97.

things being equal, a small individual of the same species of animal metabolizes absolutely less, but relatively more as compared with the unit of the weight of the body. With increasing bodily weight the total metabolism per kilo of animal diminishes, which is true first for individuals of the same species of animals, but also seems to have a certain correctness on the comparison of different species of animals. It must be remarked that the relation between flesh and fat in the body exerts an important influence. The extent of the metabolism is dependent upon the quantity of active cells, and a very fat individual therefore decomposes less substance per kilo than a lean person of the same weight. According to RUBNER [1] the importance of the size of the flesh or cell-mass in the body is overestimated. In his investigations on two boys, one of whom was corpulent and the other normally developed, and on comparing the food-need with that found by CAMERER for boys of the same weight, RUBNER came to the result that the exchange of force in the corpulent boy almost completely corresponded with that in the non-corpulent boy of the same weight. By approximately estimating the quantity of fat in the body RUBNER was also able, from the protein condition, to compare the calculated exchange of energy with that actually found. The exchange per kilo amounted to 52 calories in the lean and 43.6 calories in the fat boy, while, if the protein condition was a measure, one would expect an exchange of calories of only 35 calories for the fat person. We cannot therefore admit of a diminished activity of the cell-mass in the fat boy, but rather an increased activity. According to RUBNER it is not the flesh-mass (protein mass) alone, but its variable functional changes, which determine the extent of decomposition. In women, who generally have less body weight and a greater quantity of fat than men, the metabolism in general is smaller, and the latter is ordinarily about four-fifths that of men.

The essential reason why small animals catabolize relatively more substance than large ones, when calculated per kilo body weight, is that the bodies of smaller animals have greater surface in proportion to their mass. On this account the loss of heat is greater, which causes increased heat production, i.e., a more active metabolism. This is also the reason why young individuals of the same kind show a relatively greater metabolism than older ones. If the heat production and carbon-dioxide elimination is calculated on the unit of surface of the body, we find, on the contrary, as the experiments of RUBNER, RICHET,[2] and others show, that they vary only slightly from a certain average in individuals of different weights.

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[1] Beiträge zur Ernährung im Knabenalter, etc. Berlin, 1902.

[2] Rubner, Zeitschr. f. Biologie, 19 and 21; Richet, Arch. de Physiol., 5 (2).

According to RUBNER's rule as to the influence of the surface, which has been recently formulated by E. VOIT, the need of energy in homœothermic animals is influenced by the development of their surface when their body is given rest, medium surrounding temperature, and relatively equal protein condition. This rule applies not only to adult human beings, but also to children and growing individuals (RUBNER, OPPENHEIMER, SCHLOSSMANN and MURSCHHAUSER). The surface is the essential factor in determining the extent of exchange of energy. In order to show this we will give here, from a work of RUBNER,[1] the figures representing the quantity of heat in calories for 1 square meter of surface for twenty-four hours:

Adult, medium diet, rest	1189 calories.
Adult, medium diet, work	1399 "
Suckling	1221 "
Child with medium diet	1447 "
Aged men and women	1099 "
Women	1004 "

The variation in the caloriific values[2] found by many investigators, which is sometimes not very small, suggests the fact that the surface rule is not alone decisive for the exchange of material in resting animals. Still it is generally considered that it is of the greatest importance in metabolism.

The more active metabolism in young individuals is apparent when we measure the gaseous exchange as well as the excretion of nitrogen. As example of the elimination of urea in children the following results of CAMERER[3] are of value:

Age.	Weight of Body in Kilos.	Urea in Grams. Per Day.	Per Kilo.
1½ years	10.80	12.10	1.35
3 "	13.30	11.10	0.90
5 "	16.20	12.37	0.76
7 "	18.80	14.05	0.75
9 "	25.10	17.27	0.69
12½ "	32.60	17.79	0.54
15 "	35.70	17.78	0.50

In adults weighing about 70 kilos, from 30 to 35 grams of urea per day are eliminated, or 0.5 gram per kilo. At about fifteen years of age the destruction of proteins per kilo is about the same as in adults. The relatively greater metabolism of proteins in young individuals is explained partly by the fact that the metabolism of material in general is more active in young animals, and partly by the fact that young animals are, as a rule, poorer in fat than those full grown.

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[1] Rubner, Ernährung im Knabenalter, page 45; E. Voit, Zeitschr. f. Biologie, 41; Oppenheimer, *ibid.*, 42; Schlossmann and Murschhauser, Bioch. Zeitschr., 18 and 26.

[2] See Magnus-Levy, Pflüger's Arch., 55; Slowtzoff (u. Zunts), *ibid.*, 95.

[3] Zeitschr. f. Biologie, 16 and 20.

That young individuals show a more active metabolism than adults, follows, as above stated, principally from the relatively greater body surface in the first as compared to the latter. According to TIGERSTEDT and SONDÉN, the greater metabolism in young animals depends nevertheless, also in part, on the fact that in these individuals the decomposition in itself is more active than in older ones. The period of growth has a considerable influence on the extent of metabolism (in man), and indeed the metabolism, even when calculated on the unit of surface of body, is greater in youth than in old age. This view is strongly disputed by RUBNER. He does not deny that differences exist between young and adult individuals which may be considered as a deviation from the above rule; still these differences may, he claims, be dependent upon the work performed, the food, and the nutritive condition. MAGNUS-LEVY and FALK[1] have reported observations which support the conclusions of SONDÉN and TIGERSTEDT.

Nurslings have a behavior different from older children, as with them during the first months of life, and especially the first three days, the metabolism, calculated on the unit of surface, is strikingly low, and lower than with adults. After about two weeks it attains about the same height as adults (SCHERER, FORSTER[2]).

In old age the metabolism is very much reduced; and even when calculated upon the square meter of surface of body it is lower than in an individual of medium age.

The question as to what extent *sex* specially influences metabolism remains to be investigated. TIGERSTEDT and SONDÉN found that in the young the carbon-dioxide elimination, per kilo of body weight, as well as per square meter of body surface, was considerably greater in males than in females of the same age and the same weight of body. This difference between the sexes seems to disappear gradually, and at old age it is entirely absent. The investigations of MAGNUS-LEVY and FALK oppose these observations. They investigated by means of the ZUNTZ-GEPPERT method, not only children, but also adults and old persons of both sexes, but could not observe any positive influence of the sex upon metabolism.[3]

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[1] Tigerstedt and Sondén, Skand. Arch. f. Physiol. **6**; Rubner, l. c.; and Arch. f. Hygiene, **66**; Magnus-Levy, Arch. f. (Anat. u.) Physiol., 1899, Suppl.

[2] Cited by A. Loewy in Oppenheimer's Handb., Bd. **4**, 189.

[3] Tigerstedt and Sondén, Skand. Arch. f. Physiol., **6**; Magnus-Levy and Falk, Arch. f. (Anat. u.) Physiol., 1899, Suppl. In regard to metabolism and its relation to the phases of sexual life and especially under the influence of menstruation and pregnancy, see the investigations of A. Ver Eecke (Bull. acad. roy. de méd. de Belgique, 1897 and 1901, and Maly's Jahresber., **30** and **31**). See also Magnus-Levy in v. Noorden's Handb. d. Pathol. d. Stoffwechsels.

As the metabolism may be kept at its lowest point by absolute rest of body and inactivity of the intestinal tract, it is manifest that work and the ingestion of food have an important bearing on the extent of metabolism.

Rest and Work. During work a greater quantity of chemical energy is converted into kinetic energy, i.e., the metabolism is increased more or less on account of work.

As explained in a previous chapter (X), work, according to the generally accepted view, has no material influence on the excretion of nitrogen. It is nevertheless true that several investigators have observed, in certain cases, an increased elimination of nitrogen; this increase does not seem to be directly related to the work, but to be caused by secondary circumstances. These observations have been explained in other ways. For instance, work may, when it is connected with violent movements of the body, easily cause dyspnœa, and this last, as FRÄNKEL [1] has shown, may occasion an increase in the elimination of nitrogen, since diminution of the oxygen supply increases the protein metabolism. In other series of experiments the quantity of carbohydrates and fats in the food was not sufficient; the supply of fat in the body was decreased thereby, and the destruction of proteins was correspondingly increased. Other conditions, such as the external temperature and the weather,[2] thirst, and drinking of water, can also influence the excretion of nitrogen. The prevailing sentiment is that muscular activity has hardly any influence on the metabolism of proteins.

On the contrary, work has a very considerable influence on the elimination of carbon dioxide and the consumption of oxygen. This action, which was first observed by LAVOISIER, has later been confirmed by many investigators. PETTENKOFER and VOIT [3] have made investigations tions on a full-grown man as to the metabolism of the nitrogenous as well as of the non-nitrogenous bodies during rest and work, partly while fasting and partly on a mixed diet. The experiments were made on a full-grown man weighing 70 kilos. The results are contained in the following table:

		Consumption of				
		Proteins.	Fat.	Carbohydrates.	CO_2 Eliminated.	O Consumed.
Fasting	Rest	79	209	...	716	761
	Work	75	380	...	1187	1071
Mixed diet.	Rest	137	72	352	912	831
	Work	137	173	352	1209	980

[1] Virchow's Arch., 67 and 71. In regard to disputed views see C. Voit, Zeitschr. f. Biol., 49, and Fränkel, *ibid.*, 50.

[2] See Zuntz and Schumburg, Arch. f. (Anat. u.) Physiol., 1895.

[3] Zeitschr. f. Biologie, 2.

In these cases work did not seem to have any influence on the destruction of proteins, while the gas exchange was considerably increased.

ZUNTZ and his pupils[1] have made important investigations on the extent of the exchange of gas as a measure of metabolism during work and caused by work. These investigations not only show the important influence of muscular work on the catabolism of material, but they also indicate, in a very instructive way, the relation between the extent of metabolism of material and its utilization for work of various kinds. We can refer only to those which are of special physiological interest.

The action of muscular work on the gas exchange does not alone appear with hard work. From the researches of SPECK and others we learn that even very small, apparently quite unessential movements may increase the production of carbon dioxide to such an extent that by not observing these, as in numerous older experiments, very considerable errors may creep in. JOHANSSON[2] has also made experiments upon himself, and finds that on the production of as complete a muscular inactivity as possible the ordinary amount of carbon dioxide (31.2 grams per hour at rest in the ordinary sense) may be reduced nearly one-third, or to an average of 22 grams per hour.

The quantity of carbon dioxide eliminated during a working period is uniformly greater than the quantity of oxygen taken up at the same time, and hence a raising of the respiratory quotient was usually considered as caused by work. This rise does not seem to be based upon the character of the chemical processes going on during work, as we have a series of experiments made by ZUNTZ and his collaborators, LEHMANN, KATZENSTEIN and HAGEMANN,[3] in which the respiratory quotient remained almost wholly unchanged in spite of work. According to LOEWY[4] the combustion processes in the animal body go on in the same way in work as in rest, and a raising of the respiratory quotient (irrespective of the transient change in the respiratory mechanism) takes place only with insufficient supply of oxygen to the muscles, as in continuous fatiguing work or excessive muscular activity for a brief period, also with local lack of oxygen caused by excessive work of certain groups of muscles. This varying condition of the respiratory quotient has been explained by

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[1] See the works of Zuntz and Lehmann, Maly's Jahresber., 19; Katzenstein, Pflüger's Arch., 49; Loewy, *ibid.*; Zuntz, *ibid.*, 68; Zuntz and Slowtzoff, *ibid.*, 95; and especially the large work "Untersuch. über den Stoffwechsel des Pferdes bei Ruhe und Arbeit," Zuntz and Hagemann, Berlin, 1898; Hohenklima und Bergwanderungen by Zuntz, Loewy, Müller and Caspari, which also contains a bibliography.

[2] Nord. Med. Arkiv. Festband, 1897; also Maly's Jahresber., 27; Speck, "Physiol. des menschl. Atmens," Leipzig, 1892.

[3] See footnote 1.

[4] Pflüger's Arch., 49.

KATZENSTEIN by the statement that during work two kinds of chemical processes act side by side. The one depends upon the work which is connected with the production of carbon dioxide, also in the absence of free oxygen, while the other brings about the regeneration which takes place by the taking up of oxygen. When these two chief kinds of chemical processes make the same progress the respiratory quotient remains unchanged during work; if by hard work the decomposition is increased as compared with the regeneration, then a raising of the respiratory quotient takes place. If, on the contrary, moderate work is continued and performed in a way so that irregularities and occasional changes in the circulation and respiration are excluded or are without importance, then the respiratory quotient may correspondingly remain the same during work as in rest. Its extent is thus determined in the first place by the nutritive material at its disposal (ZUNTZ and his pupils).

The theory of LOEWY and ZUNTZ, that the raising of the respiratory quotient during work is to be explained by an insufficient supply of oxygen, is opposed by LAULANIÉ.[1] He has observed the reverse, namely, a diminution in the respiratory quotient during continuous excessive work, and this is not reconcilable with the above statements. He considers that sugar is the source of muscular energy, and that the rise in the respiratory quotient is due to an increased combustion of sugar. Its diminution, he explains, is caused by a re-formation of sugar from fat which takes place at the same time and is accompanied by an increased consumption of oxygen.

In *sleep* metabolism decreases as compared with that during waking hours, and the most essential reason for this is the muscular inactivity during sleep. The investigations of RUBNER upon a dog, and of JOHANSSON[2] upon human beings, teach us that if the muscular work is eliminated the metabolism during waking hours is not greater than in sleep.

The action of *light* also stands in close connection with the question of the action of muscular work. It seems positively proven that metabolism is increased under the influence of light. Most investigators, such as SPECK, LOEB, and EWALD,[3] consider that this increase is due to the movements caused by the light or an increased muscle tonus, and in man an increase in metabolism under the influence of light with complete rest has not been observed. Divergent results have been obtained in animals, and our knowledge of the truth is not yet complete.[4]

Mental activity does not seem to have any influence on metabolism according to the means at hand for studying this influence.

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[1] Arch. de Physiol. (5), 8, 572.

[2] Rubner, Ludwig-Festschr., 1887; Loewy, Berl. klin. Wochenschr., 1891, 434; Johansson, Skand. Arch. f. Physiol., 8.

[3] Speck, l. c.; Loeb, Pflüger's Arch., 42; Ewald, Journ. of Physiol., 18.

[4] See larger handbooks for the literature on this question.

The Action of the External Temperature also stands in close relation to muscular work, namely to the question as to whether the chemical heat regulation is independent of the muscular activity. The heat regulation, as is well known, is of two kinds, namely the *chemical heat regulation*, which consists in a change in the metabolism and which manifests itself as an increased heat production due to the increased metabolism at low temperatures, and the *physical heat regulation*, which occurs generally at higher temperatures and is caused by changes in the conditions in the heat elimination of the thermal equilibrium.

In regard to the chemical heat regulation, which will only be discussed here, we must differentiate between cold-blooded and warm-blooded animals. In the first the metabolism rises with an increase in the surrounding temperature, while in the second group the conditions are different. The experiments of SPECK, LOEWY and JOHANSSON [1] on human beings have shown that the lowering of the external temperature is without influence upon the extent of metabolism (measured by the gas exchange) only as long as all natural and non-voluntary movements of the muscles are excluded; otherwise the metabolism is raised. A chemical heat regulation, i.e., a rise in metabolism without noticeable movements of the muscles, is not accepted in man, or at least it has not been proven. The heat regulation, in man, at lower temperatures seems to be brought about by the natural or reflex production of muscle action, nor has a chemical heat regulation in the reverse sense, namely, a fall in the catabolism by raising the external temperature, been shown in man. The investigations of EYKMAN [2] upon inhabitants of the tropics also show the same result, namely, that in human beings no appreciable chemical heat regulation occurs.

In animals the conditions are different so far as that a chemical heat regulation in the true sense has been positively shown. The investigations of RUBNER [3] on various animals have shown that the reduction of the external temperature with these, causes a considerable chemical heat regulation by increasing the metabolism without any chill or shiver movements. On sufficient cooling the temperature of the body may fall irrespective of the increased metabolism, and at a certain limit of body temperature the exchange of material becomes still lower with decreasing temperature. According to RUBNER many animals can bear a temperature of 0° C. for days in absolute rest. If the natural muscular activity is eliminated by poisoning with curare or by section of the spinal cord, then, as shown by PFLÜGER [4] and his pupils, the warm-blooded animal behaves

[1] Speck, l. c.; Loewy, Pflüger's Arch., **46**; Johansson, Skand. Arch. f. Physiol., **7**.
[2] Virchow's Arch., **133**, and Pflüger's Arch., **64**.
[3] Arch. f. Hyg., **37**, and Handbuch d. Hyg., Bd. **1**, Leipzig, 1911.
[4] See footnote 2, page 591.

like a cold-blooded animal, and the metabolism decreases parallel with the body temperature. In normal animals, on the contrary, the body temperature can be kept constant, on lowering the external temperature, by an increased metabolism; but also in such animals because of a rise in the external temperature a rise in the metabolism above a certain limit can also take place.

A very interesting and important question is the action of high altitude upon the oxidation processes, the economy of temperature, the protein exchange and the general metabolism. The results of the laborious and important investigations on this subject may be found in the large work of N. ZUNTZ, A. LOEWY, F. MÜLLER and W. CASPARI.[1]

That the *ingestion of food* raises the metabolism has been known for a rather long time, and this has been studied by ZUNTZ, v. MERING, MAGNUS-LEVY, VOIT, RUBNER, JOHANSSON and collaborators, also by HEILNER and by GIGON.[2] It follows from these investigations that this rise in metabolism, which in man, on sufficient supply of food, amounts to a rise of 10–15 per cent of the basal requirement and with abundant supply of food may be still larger (35 per cent in the researches of JOHANSSON, TIGERSTEDT and collaborators), has a double cause, namely, partly a digestion work (ZUNTZ) and partly a chemical decomposition (specific dynamic action of RUBNER) which takes place at the same time.

The sum of all the work which is necessary for the chemical transformation of the foods, as well as for the mechanical division and transportation of the food in the intestinal canal, is called the *digestion work* by ZUNTZ. That such work exists has been shown by ZUNTZ and v. MERING by comparative tests of the different action upon metabolism by foods introduced per os and intravenously, and recently COHNHEIM[3] has shown that in sham feeding an increased catabolism of non-nitrogenous body constituents took place. The influence of digestion work in ZUNTZ's sense is especially apparent in herbivora, in which this work, according to ZUNTZ and collaborators, may amount to the consumption of more than 50 per cent of the total energy content of the raw fodder.

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[1] Hohenklima und Bergwanderungen in ihrer Wirkung auf den Menschen, Berlin, 1906.

[2] Zuntz and v. Mering, Pflüger's Arch., 15; Zuntz, Naturw. Rundschau, 21 (1906), with Hagemann, l. c., with Magnus-Levy, Pflüger's Arch., 49; Magnus-Levy, *ibid.*, 55, and v. Noorden's Handbuch; Voit, Hermann's Handbuch, 6; Rubner, Zietschr. f. Biol., 19 and 21; and Arch. f. Hyg., 66; Johansson, Skand. Arch. f. Physiol., 21, with Koraen, *ibid.*, 18; Heilner, Zeitschr. f. Biol., 48 and 50; Gigon, Pflüger's Arch., 140.

[3] Arch. f. Hyg., 57.

On partaking of large amounts of food, especially proteins, by carnivora, the digestion work in the above sense is not sufficient to account for the increase in metabolism, and in these cases, besides this, we must accept an increase in the chemical transformation process in the animal body brought on by the foodstuffs in an unknown manner (*specific dynamic action* of foodstuffs, according to RUBNER). The only real difference in opinion between the various experimenters consists, so far as HAMMARSTEN can see, in that according to the ZUNTZ school, normally on supplying sufficient food it is the digestion work in the above sense which chiefly causes the rise in metabolism after taking food, while according to the views of VOIT-RUBNER, with which HEILNER agrees, it is on the contrary the specific dynamic action.

That the proteins or their cleavage products, without regard to the digestion work, cause a rise in the metabolism seems to be generally accepted. This rise, according to GIGON, is not proportional to the protein supply, as on supplying quantities of protein represented by 1:2:4:3 the oxygen absorption was in the proportion 1:3:6:9 and the carbon dioxide elimination was in the proportion 1:4:8:12. On the introduction of glucose GIGON found, as first shown by JOHANSSON,[1] that the introduction of carbohydrate caused a proportional rise in the carbon dioxide elimination to a maximal limit of 150 grams. The conditions on supplying fat are harder to judge, but GIGON found no rise in metabolism on introducing oil.

The rise in the gas exchange occurring after feeding protein and sugar is added, according to GIGON, entirely to the basal metabolism. A substitution in the basal metabolism of the catabolized body constituents by the food taken does not take place according to GIGON and, as example, the protein is not replaced from catabolism by the sugar introduced. The isodynamic law does not apply to the metabolism occurring the first few hours after supplying food, as shown by JOHANSSON and HELLGREN, and GIGON [2] believes that the foodstuffs first pass into the various depots of the body to be later used for purposes of energy. Proteins serve only to a slight degree to replace the catabolized body protein; the remainder is stored up in part as glycogen and in part as fat. The fat is deposited as such and the carbohydrates are deposited as glycogen and fat.

As the three foodstuffs influence the metabolism in very different ways we can, according to GIGON, speak of a specific action of the foodstuffs. This action, according to him, is more of a material than of a dynamic kind, and the expression, specific dynamic action, may lead to an erroneous conception.

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[1] Skand. Arch. f. Physiol., 21.

[2] Johansson with Hellgren, Hammarsten's Festschrift, 1906; Gigon, Skand. Arch. f. Physiol., 21.

V. THE NECESSITY OF FOOD BY MAN UNDER VARIOUS CONDITIONS.

Various attempts have been made to determine the daily quantity of organic food needed by man. Certain investigators have calculated from the total consumption of food by a large number of similarly fed individuals—soldiers, sailors, laborers, etc.—the average quantity of foodstuffs required per head. Others have calculated the daily demand for food from the quantity of carbon and nitrogen in the excreta, or calculated it from the exchange of force of the persons experimented upon. Others, again, have calculated the quantity of nutritive material in a diet by which an equilibrium was maintained in the individual for one or several days between the consumption and the elimination of carbon and nitrogen. Lastly, still others have quantitatively determined, during a period of several days, the organic foodstuffs daily consumed by persons of various occupations who chose their own food, by which they were well nourished and rendered fully capable of work.

Among these methods a few are not quite free from objection, and others have not as yet been tried on a sufficiently large scale. Nevertheless the experiments collected thus far serve, partly because of their number and partly because the methods correct and control one another, as a good starting-point in determining the diet of various classes and similar questions.

If the quantity of foodstuffs taken daily be converted into calories produced during physiological combustion, we then obtain some idea of the sum of the chemical energy which under varying conditions is introduced into the body. It must not be forgotten that the food is never completely absorbed, and that undigested or unabsorbed residues are always expelled from the body with the feces. The gross results of calories calculated from the food taken must therefore, according to RUBNER, be diminished by at least 8 per cent. This figure is true at least when the human being partakes of a mixed diet of about 60 per cent of the proteins as animal, and about 40 per cent of the proteins as vegetable foodstuffs. With more one-sided vegetable food, especially when this is rich in undigestible cellulose, a much larger quantity must be subtracted.

The following summary contains a few examples of the quantity of food which is consumed by individuals of various classes of people under different conditions. In the last column we also find the quantity of living force which corresponds to the quantity of food in question, calculated as calories, with the above-stated correction. The calories are therefore net results, while the figures for the nutritive bodies are gross results.

	Proteins.	Fat.	Carbohydrates.	Calories.	Authority.
Soldier during peace.....	119	40	529	2784	PLAYFAIR.[1]
Soldier light service.....	117	35	447	2424	HILDESHEIM.
Soldier in field.....	146	46	504	2852	HILDESHEIM.
Laborer.....	130	40	550	2903	MOLESCHOTT.
Laborer at rest.....	137	72	352	2458	PETTENKOFER and VOIT.
Cabinetmaker (40 years).	131	68	494	2835	FORSTER.[2]
Young physician.....	127	89	362	2602	FORSTER.
Young physician.....	134	102	292	2476	FORSTER.
Laborer (36 years).....	133	95	422	2902	FORSTER.
English smith.....	176	71	666	3780	PLAYFAIR.
English pugilist.....	288	88	93	2189	PLAYFAIR.
Bavarian wood-chopper..	135	208	876	5589	LIEBIG.
Laborer in Silesia.....	80	16	552	2518	MEINERT.[3]
Seamstress in London...	54	29	292	1688	PLAYFAIR.
Swedish laborer.....	134	79	485	3019	HULTGREN and LANDERGREN.[4]
Japanese student.....	83	14	622	2779	EIJKMAN.[5]
Japanese shopman.....	55	.6	394	1744	TAWARA.[6]

We have a very large number of complete investigations upon the diet of people of different vocations in America, but they are too extensive to enter into, hence we must refer to the original publications of ATWATER.[6]

It is evident that persons of essentially different weight of body who live under unequal external conditions must need essentially different food. It is also to be expected (and this is confirmed by the table) that not only the absolute quantity of food consumed by various persons, but also the relative proportion of the various organic nutritive substances, shows considerable variation. Results for the daily need of human beings in general cannot be given. For certain classes, such as soldiers, laborers, etc., results may be given which are valuable for the calculation of the daily rations.

Based on extensive investigations and a very wide experience, VOIT has proposed the following average quantities for the daily diet of adults:

	Proteins.	Fat.	Carbohydrates.	Calories.
For men.....	118 grams	56 grams	500 grams	2810

But it should be remarked that these data relate to a man weighing 70 to 75 kilos and who was engaged daily for ten hours in not too fatiguing labor.

The quantity of food required by a woman engaged in moderate work

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[1] In regard to the earlier researches cited in this table, we refer the reader to Voit, in Hermann's Handbuch, **6**, 519.

[2] *Ibid.*, and Zeitschr. f. Biologie, **9**.

[3] Armee- und Volksernährung, Berlin, 1880.

[4] Untersuching über die Ernährung schwedischer Arbeiter bei frei gewählter Kost, Stockholm, 1891. Maly's Jahresber., **21**.

[5] Cited from Kellner and Mori in Zeitschr. f. Biologie, **25**.

[6] Report of the Storrs Agric. Expt. Station, Conn., 1891–1895, and 1896, and U. S. Depart. of Agriculture, Bull. **53**, 1898.

is about four-fifths that of a laboring man, and we may consider the following as a daily diet with moderate work:

	Proteins.	Fat.	Carbohydrates.	Calories.
For women.....	94 grams	45 grams	400 grams	2240

The proportion of fat to carbohydrates is here as 1:8–9. Such a proportion often occurs in the food of the poorer classes who chiefly live upon the cheap and voluminous vegetable food, while this ratio in the food of wealthier persons is 1:3–4. It would be desirable if in the above rations the fat were increased at the expense of the carbohydrates, but unfortunately on account of the high price of fat such a modification cannot always be made.

In examining the above figures for the daily rations it must not be forgotten that those for the various foodstuffs are gross results. They consequently represent the quantity of those which must be taken in, and not those which are really absorbed. The figures for the calories are, on the contrary, net results.

The various foods are, as is well known, not equally digested and absorbed, and in general the vegetable foods are less completely consumed than animal foods. This is especially true of the proteins. When, therefore, VOIT, as above stated, calculates the daily quantity of proteins needed by a laborer as 118 grams, he starts with the supposition that the diet is a mixed animal and vegetable one, and also that of the above 118 grams about 105 grams are absorbed. The results obtained by PFLÜGER and his pupils BOHLAND and BLEIBTREU [1] on the extent of the metabolism of proteins in man with an optional and sufficient diet correspond well with the above figures, when the unequal weight of body of the various persons experimented upon is sufficiently considered.

As a rule, the more exclusively a vegetable food is employed, the smaller is the quantity of proteins in it. The strictly vegetable diet of certain people, as that of the Japanese and of the so-called vegetarians, is therefore a proof that, if the quantity of food be sufficient, a person may exist on considerably smaller quantities of proteins than VOIT suggests. It follows from the investigations of HIRSCHFELD, KUMAGAWA and KLEMPERER, SIVÉN, and others (see pages 903, 915) that an almost complete or indeed a complete nitrogenous equilibrium may be attained by the sufficient administration of non-nitrogeneous nutritive bodies with relatively very small quantities of proteins.

If we bear in mind that the food of people of different countries varies greatly, and that the individual also takes essentially different nourishment according to the external conditions of living and the influence of climate, it is not remarkable that a person accustomed to a mixed

[1] Bohland, Pflüger's Arch., **36**; Bleibtreu, *ibid.*, **38**.

diet can exist for some time on a strictly vegetable diet deficient in proteins. No one doubts the ability of man to adapt himself to a heterogeneously composed diet when this is not too difficult of digestion and is sufficient in quantity; nor can we deny that it is possible for a man to exist for a long time with smaller amounts of protein than VOIT suggests, namely 118 grams. Thus O. NEUMANN [1] experimented on himself during 746 days in three series of experiments, and his diet consisted of 74.2 grams protein, 117 grams fat, and 213 grams carbohydrates = 2367 gross calories, with a weight of 70 kilos and with ordinary laboratory work. These figures cannot be compared with those obtained by VOIT's worker, weighing 70 kilos, whose work was harder than a tailor's and easier than a blacksmith's; for example, the work of a mason, carpenter, or cabinet-maker. The very extensive investigations recently performed by CHITTENDEN [2] on the determination of the extent of protein necessary are of great interest. These investigations, upon a total of twenty-six persons, extended over a period of five to twenty months, and consisted of careful observations upon the manner of living, food taken, nitrogen elimination, and the ability of performing work. The individuals were divided into three groups. The first consisted of five professional men (four assistants and one professor). The second group was composed of thirteen soldiers (of the sanitary corps of the United States army) who besides their daily work were given gymnastic exercises for six months. The third group consisted of eight athletic students who were trained in different kinds of sport.

In all the persons experimented upon the original nitrogen content of the food, which corresponded to VOIT's value or were somewhat higher, was gradually reduced more or less. The total calories supplied were not increased above the original value, but rather diminished to a reasonable extent. The bodily as well as the mental ability was repeatedly tested. As it is not possible to enter into the details of the investigation the following will be sufficient to show the results. With a diet corresponding to VOIT'S values the amount of urine nitrogen per day is 16 grams, corresponding to a total protein catabolism in the body of 100 grams, or 1.43 grams per kilo. The corresponding results for the above three groups may be found in the following table, where for comparison HAMMARSTEN also includes the figures for VOIT'S diet:

	Urine Nitrogen.		Catabolised Protein.		Protein per Kilo.	
	Min.	Max.	Min.	Max.	Min.	Max.
Group 1	5.69	8.99	35.6	56.19	0.61	0.86
Group 2	7.03	8.39	43.9	52.44	0.74	0.87
Group 3	7.47	11.06	46.7	69.10	0.75	0.92
Voit's figures	16		100		1.43	

[1] Arch. f. Hygiene, 45.

[2] R. H. Chittenden, Physiological Economy in Nutrition, New York, 1904.

The chief results from these investigations are that on partaking of amounts of protein much smaller than VOIT'S figures, without changing the original supply of calories and indeed diminishing the same, the persons experimented upon remained not only in nitrogenous equilibrium, but in perfect health, and were not only able to perform ordinary work, but were indeed regularly able to perform much greater work.

From these investigations, which extended over a long period and were carried on with special care in exactitude, it cannot be denied that man can for a long time exist with much smaller quantities of protein than VOIT'S figures call for, which is also derived from the experience of vegetarians, and from people living almost entirely upon vegetable food. On the other hand it must not be forgotten that VOIT'S figures represent average results not theoretically necessary, but which have been shown to be the actual diet developed from habit, custom, conditions of life and climate, with sufficient nourishment and free selection for centuries in Middle and North Europe. A rational change in this food requirement based upon scientific facts is just as difficult to determine as it is to carry out practically. Certain standard figures for the general needs of nutrition cannot be established because the conditions in various countries are different and must necessarily be so. The numerous compilations (of ATWATER and others [1]) on the diet of different families in America have given the figures 97–113 grams protein for a man, and the very careful investigations of HULTGREN and LANDERGREN have also shown that the laborer in Sweden with moderate work and an average body weight of 70.3 kilos, with optional diet, partakes 134 grams protein, 79 grams fat, and 522 grams carbohydrates. The quantity of protein is here greater than is necessary, according to VOIT. On the other hand LAPICQUE [2] found 67 grams protein for Abyssinians and 81 grams for Malaysians (per body weight of 70 kilos), materially lower figures.

If we compare the figures on page 933 with the average figures proposed by VOIT for the daily diet of a laborer, it would seem at the first glance as if the food consumed in certain cases was considerably in excess of the need, while in other cases, as, for instance, that of a seamstress in London, it was entirely insufficient. A positive conclusion cannot, therefore, be drawn if we do not know the weight of the body, as well as the labor performed by the person, and also the conditions of living.

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[1] Atwater, Report of the Storrs Agric. Expt. Station, Conn., 1891–1895 and 1896; also Nutrition investigations at the University of Tennessee, 1896 and 1897; U.S. Dept. of Agriculture, Bull. 53, 1898. See also Atwater and Bryant, *ibid.*, Bull. 75. Jaffa, *ibid.*, 84; Grindley, Sammis, and others, *ibid.*, 91.

[2] Hultgren and Landergren, l. c.; Lapicque, Arch. de Physiol. (5), 6.

It is certainly true that the amount of nutriment required by the body is not directly proportional to the body weight, for a small body consumes relatively more substance than a larger one, and varying quantities of fat may also cause a difference; but a large body, which must maintain a greater quantity, consumes an absolutely greater amount of substance than a small one, and in estimating the nutritive need one must also always consider the weight of the body. According to VOIT, the diet for a laborer with 70 kilos body weight requires 40 calories for each kilo. EKHOLM[1] calculates, basing it upon his experiments, that for a man weighing 70 kilos, busied with reading and writing, the net calories are 2450 and the gross calories 2700, or 35 and 38.6 calories per kilo. In the ordinary sense for a resting man, the general food requirement is calculated in round numbers as 30 calories for every kilo. The minimum figure for metabolism during sleep and in as complete rest as possible has been found by SONDÉN, TIGERSTEDT and JOHANSSON[2] to be 24–25 calories.

As several times stated above, the demands of the body for nourishment vary with different conditions of the body. Among these conditions two are especially important, namely, work and rest.

In a previous chapter, in which muscular labor was spoken of, it was seen that all foodstuffs have almost the same power of serving as a source of muscular work, and that the muscles, it seems, select that foodstuff which is supplied to them in the greatest quantity. As a natural sequence it is to be expected that muscular activity requires indeed an increased supply of foodstuffs, but no essential change in their relation as compared to rest.

Still this does not seem to hold true in daily experience. It is a well-known fact that hard-working individuals—men and animals—require a greater quantity of proteins in the food than less active ones. This contradiction, is however, only apparent, and it depends, as VOIT has shown, upon the fact that individuals used to violent work are more muscular. For this reason a person performing severe muscular labor requires food containing a larger proportion of proteins than an individual whose occupation demands less violent exertion. Another fact is that the diet rich in proteins is often concentrated and less bulky, and also that in many cases of training, a diet yielding as little fat as possible is selected.

If we compare the results for the needs of food in work and rest which are obtained under conditions which can be readily controlled, it is found that the above statements are in general confirmed. As example of this

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[1] Skand. Arch. f. Physiol., 11.

[2] Sondén and Tigerstedt, Skand. Arch. f. Physiol., 6; Johansson, *ibid.*, 7; Tigerstedt, Nord. Med. Arkiv. Festband., 1897.

the following tables give the rations of soldiers in peace and in the field and the average figures from the detailed data of various countries:[1]

	A. Peace Ration.			B. War Ration.		
	Proteins.	Fat.	Carbohydrates.	Proteins.	Fat.	Carbohydrates.
Minimum...	108	22	504	126	38	484
Maximum...	165	97	731	197	95	688
Mean.....	130	40	551	146	59	557

The following figures for the daily ration are obtained from the above averages:

	Proteins.	Fat.	Carbohydrates.	Calories.
In peace.....	130	40	551	2900
In war.....	146	59	557	3250

If we calculate the fat in its equivalent quantity of starch, then the relation of the proteins to the non-nitrogenous foods is:

In peace.....	1 : 4.97
In war.....	1 : 4.79

The relation in both cases is nearly the same. Similar results are obtained when we start with VOIT's figures for a soldier in manœuver A (hard work) and B (strenuous work) in war.

	Proteins.	Fat.	Carbohydrates.	Calories.
A.....	135	80	500	3013
B.....	145	100	500	3218

The relation here, when the fat is recalculated as starch, in both cases is the same, or equal to 1:5.

If we calculate that portion of the total calories supplied which falls to each group of the foodstuffs, it is found that 16–19 per cent comes from the protein in rest as well as with medium and strenuous work. For the fat and the carbohydrates the variations are greater; the chief quantity of calories comes from the carbohydrates. Of the total calories 16–30 per cent comes from the fat and 50–60 per cent from the carbohydrates.

The importance of the food-demand for working individuals is shown by the figures given on page 933 for a wood-chopper in Bavaria. A need of more than 4000 calories occurs but seldom, and with very hard work the demand may rise even to 7000 calories (ATWATER and BRYANT, JAFFA[2]).

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[1] Germany, Austria, Switzerland, France, Italy, Russia, and the United States. It is not known by the author whether these figures have been changed in the last few years in the various countries, and hence whether they must be modified or not.

[2] See footnote 1, page 936.

As more work requires an increase in the absolute quantity of food, so the quantity of food must be diminished when little work is performed. The question as to how far this can be done is of importance in regard to the diet in prisons and poorhouses. We give below the following as example of such diets:

	Proteins.	Fat.	Carbohydrates.	Calories.	
Prisoner (not working)..	87	22	305	1667	SCHUSTER.[1]
Prisoner (not working)..	85	30	300	1709	VOIT.
Man in poorhouse.....	92	45	332	1985	FORSTER.[2]
Woman in poorhouse...	80	49	266	1724	FORSTER.

The figures given by VOIT are, he says, the lowest reported for a non-working prionser. He considers the following as the lowest diet for old non-working people:

	Proteins.	Fat.	Carbohydrates.	Calories.
Men.....	90	40	350	2200
Women.....	80	35	300	1723

In calculating the daily diet it is in most cases sufficient to ascertain how much of the various foodstuffs must be administered to the body in order to keep it in the proper condition to perform the work required of it. In other cases it may be a question of improving the nutritive condition of the body by properly selected food; and there are also cases in which it is desired to diminish the mass or weight of the body by an insufficient nutrition. This is especially the case in obesity, and all the dietaries proposed for this purpose are chiefly starvation cures, which is readily apparent if we study such dietaries.

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[1] See Voit, Unters. der Kost, München, 1877, page 142. See also Hirschfeld, Maly's Jahresber., 30.

[2] *Ibid.*, page 186.

TABLE I.—FOODS.[1]

1. Animal Foodstuffs.	1000 Parts contain						Relation of 1 : 2 : 3.		
	1 Proteids and Extractives.	2 Fat.	3 Carbohydrates.	4 Ash.	5 Water.	6 Waste.	1	:2	:3
a. MEAT WITHOUT BONES.									
Fat beef [2]	183	166		11	640		100	90	0
Beef (average fat [1])	196	98		18	688		100	50	0
Beef [3]	190	120		18	672		100	63	0
Corned beef (average fat)	218	115		117	550		100	53	0
Veal	190	80		13	717		100	42	0
Horse, salted and smoked	318	65		125	492		100	20	0
Smoked ham	255	365		100	280		100	143	0
Pork, salted and smoked [3]	100	660		40	130		100	660	0
Meat from hare	233	11		12	744		100	5	0
" " chicken	195	93		11	701		100	48	0
" " partridge	253	14		14	719		100	6	0
" " wild duck	246	31		12	711		100	13	0
b. MEAT WITH BONES.									
Fat beef [2]	156	141		9	544	150	100	90	0
Beef (average fat [1])	167	83		15	585	150	100	49	0
Beef, slightly corned	175	93		85	480	167	100	53	0
Beef, thoroughly corned	190	100		100	430	180	100	53	0
Mutton, very fat	135	332		8	437	88	100	246	0
" average fat	160	160		10	520	150	100	100	0
Pork, fresh, fat	100	460		5	365	70	100	460	0
" corned, fat	120	540		60	200	80	100	450	0
Smoked ham	200	300		70	340	90	100	150	0
c. FISHES.									
River eel, fresh, entire	89	220		6	352	333	100	246	0
Salmon, " "	121	67		10	469	333	100	56	0
Anchovy, " "	128	39		11	489	333	100	31	0
Flounder, " "	145	14		11	580	250	100	9	0
River perch, fresh, entire	100	2		8	440	450	100	2	0
Torsk " "	86	1		8	455	450	100	1	0
Pike, " "	82	1		6	461	450	100	1	0
Herring, salted, entire	140	140		100	280	340	100	100	0
Anchovy, " "	116	43		107	334	400	100	37	0
Salmon (side), salted	200	108		132	460	100	100	54	0
Kabeljau (salted haddock)	246	4		178	472	100	100	1	0
Codfish (dried ling)	532	5		106	257	100	100	1	0
" (dried torsk)	665	10		59	116	150	100	1	0
Fish-meal from variety of GADUS	736	7		87	170		100	1	0

[1] The results in the following tables are chiefly compiled from the summary of ALMÉN and of KÖNIG. We here designate as "waste" that part of the foods which is lost in the preparation or that which is not used by the body; for instance, bones, skin, egg-shells, and the cellulose vegetable foods.

[2] Meat such as is ordinarily sold in the markets in Sweden.

[3] Pork, chiefly from the breast and belly, such as occurs in the rations of Swedish soldiers.

TABLE I.—FOODS—(*Continued*).

1. Animal Foodstuffs.	1000 Parts contain						Relation of 11 : 2 : 3.		
	1 Proteids and Extractive.	2 Fat.	3 Carbohydrates.	4 Ash.	5 Water.	6 Waste.	1	:2	:3
d. Inner Organs (Fresh).									
Brain	116	103		11	770		100	89	6
Beef-liver	196	56	11	17	720		100	28	0
Beef-heart	184	92		10	714		100	50	0
Heart and lungs of mutton	163	106		10	721		100	65	0
Veal-kidney	221	38		13	728		100	17	0
Ox tongue (fresh)	150	170		10	670		100	113	0
Blood from various animals (average results)	182	2		9	807		100	1	0
e. Other Animal Foods.									
Variety of pork-sausage (Mettwurst)	190	150		50	610		100	79	0
Same for frying	220	160		55	565		100	73	0
Butter	7	850	7	15	119		100	12100	100
Lard	3	990			7		100	33000	0
Meat extract	304			175	217				
Cow's milk (full)	35	35	50	7	873		100	100	143
" " (skimmed)	35	7	50	7	901		100	20	143
Buttermilk	41	9	38	7	905		100	22	93
Cream	37	257	35	6	665		100	695	95
Cheese (fat)	230	270	40	60	400		100	117	17
" (poor)	334	66	50	50	500		100	19	15
Whey cheese (poor)	89	70	456	56	329		100	79	512
Hen's egg, entire	106	93	4	8	654	135	100	88	4
" " without shell	122	107	5	10	756		100	88	4
Yolk of egg	160	307		13	520		100	192	0
White of egg	103	7	7	8	875		100	7	7
2. Vegetable Foodstuffs.									
Wheat (grains)	123	17	676	18	140	26	100	14	549
Wheat-flour (fine)	110	10	740	8	120	12	100	11	654
" " (very fine)	92	11	768	3	120	6	100	12	835
Wheat-bran	150	39	439	50	130	192	100	26	292
Wheat-bread (fresh)	88	10	550	17	330	5	100	11	625
Macaroni	90	3	768	8	131		100	3	853
Rye (grains)	115	17	688	18	140	22	100	15	600
Rye-flour	115	15	720	20	110	20	100	13	626
Rye-bread (dry)	114	20	725	15	110	16	100	18	634
" (fresh, coarse)	77	10	480	16	409	17	100	14	623
" (fresh, fine)	80	14	514	11	370	11	100	18	634
Barley (grains)	111	21	654	26	140	48	100	19	589
Scotch barley	110	10	720	7	146	7	100	9	654
Oat (grains)	117	60	563	30	130	100	100	51	481
" (peeled)	140	60	660	20	100	20	100	43	471
Corn	101	58	656	17	140	28	100	57	662
Rice (peeled for boiling)	70	7	770	2	146	5	100	19	1100
French beans	232	21	537	36	137	37	100	9	231
Peas (yellow or green, dry)	220	15	530	25	150	60	100	7	240
Flour from peas	270	15	520	25	125	45	100	6	192

TABLE I.—FOODS—(*Continued*).

2. Vegetable Foodstuffs.	1000 Parts contain						Relation of 1 : 2 : 3.		
	1 Proteids and Extractives.	2 Fat.	3 Carbohydrates.	4 Ash.	5 Water.	6 Waste.	1	:2	:3
Potatoes	20	2	200	10	760	8	100	10	1030
Turnips	14	2	74	7	893	10	100	14	529
Carrot (yellow)	10	2	90	10	873	15	100	20	900
Cauliflower	25	4	50	8	904	9	100	16	200
Cabbage	19	2	49	12	900	18	100	11	258
Beans	27	1	66	6	888	12	100	4	244
Spinach	31	5	33	19	908	8	100	16	106
Lettuce	14	3	22	10	944	7	100	21	157
Cucumbers	10	1	23	4	956	6	100	10	230
Radishes	12	1	38	7	934	8	100	8	317
Edible mushrooms (average)	32	4	60	9	877	18	100	12	188
Same dried in the air (average)	219	25	412	61	160	123	100	12	188
Apples and pears	4		130	3	832	31	100		3250
Various berries (average)	5		90	6	849	50	100		1800
Almonds	242	537	72	29	54	66	100	222	30
Cocoa	140	480	180	50	55	95	100	343	129

TABLE II.—MALT LIQUORS

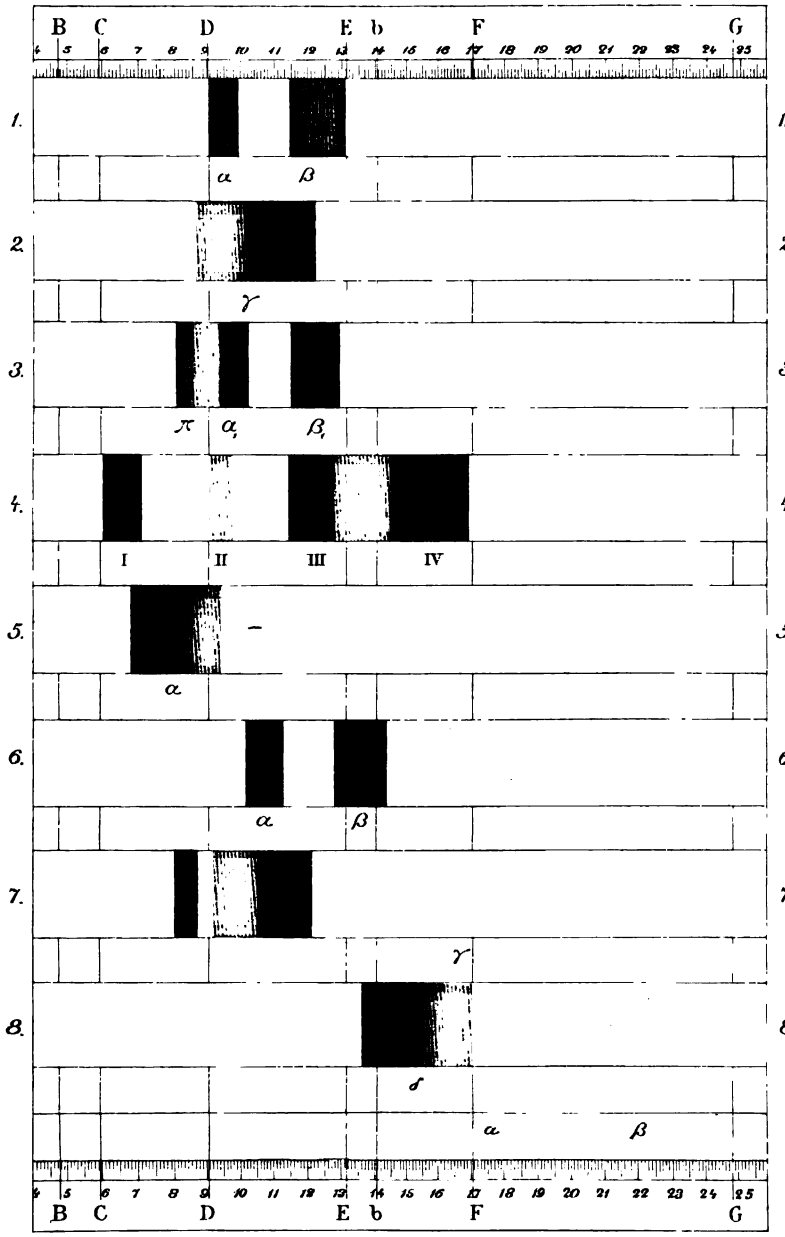
1000 Parts by Weight contain	Water.	Carbon Dioxide.	Alcohol.	Extract.	Proteids.	Sugar.	Dextrin.	Acids.	Glycerine.	Ash.
Porter	871	2	54	76	7	13		3.0	—	4
Beer (Swedish)	887		28	—	15	65		—	—	5
" (Swedish export)	885		32	—	7	73		—	—	3
Draught-beer	911	2	35	55	8	10	31	2.9	2	2
Lager-beer	903	2	40	58	4	7	47	1.5	2	2
Bock-beer	881	2	47	72	6	13	—	1.7	—	3
Weiss-beer	916	3	25	59	5	—	—	4.0	—	2
Swedish "Svagdricka"	945	—	22	—	7	23		—	—	3

TABLE III.—WINES AND OTHER ALCOHOLIC LIQUORS

1000 Parts by Weight contain	Water.	Alcohol, Vol. Per Cent.	Extract.	Sugar.	Acid and Potassium Bitartrate.	Glycerine.	Ash.	Carbon Dioxide, Vol. Per Cent.
Bordeaux wine	883	94	23	6	5.9	—	2.0	
White wine (Rheingau)	863	115	23	4	5.0	—	2.0	
Champagne	776	90	134	115	6.0	1.0	1 0	60–70
Rhine wine (sparkling)	801	94	105	87	6.0	1.0	2.0	
Tokay	808	120	72	51	7.0	9.0	3.0	
Sherry	795	170	35	15	5.0	6.0	5.0	
Port wine	774	164	62	40	4.0	2.0	3.0	
Madeira	791	156	53	33	5.0	3.0	3.0	
Marsala	790	164	46	35	5.0	4.0	4.0	
Swedish punch	479	263	—	332	—	—	—	
Brandy	—	460	—	—	—	—	—	
French cognac	—	550	—	—	—	—	—	
Liqueurs	—	442–590	—	260–475	—	—	—	

SPECTRUM PLATE

1. Absorption spectrum of a solution of *oxyhæmoglobin*.
2. Absorption spectrum of a solution of *hæmoglobin*, obtained by the action of an ammoniacal ferro-tartrate solution on an oxyhæmoglobin solution.
3. Absorption spectrum of a faintly alkaline solution of *methæmoglobin*.
4. Absorption spectrum of a solution of *hæmatin* in ether containing oxalic acid.
5. Absorption spectrum of an alkaline solution of *hæmatin*.
6. Absorption spectrum of an alkaline solution of *hæmochromogen*, obtained by the action of an ammoniacal ferro-tartrate solution on an alkaline-hæmatin solution
7. Absorption spectrum of an acid solution of *hæmatoporphyrin*.
8. Absorption spectrum of an ammoniacal solution of *urobilin* after the addition of a zinc-chloride solution.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INDEX OF AUTHORS

Abderhalden, E., enzymes, 50, 53, 54, 62, 65, 525; protein hydrolysis, 84, 106, 107, 109, 114, 121–125, 132, 133, 142, 146, 154, 262, 288, 507, 605, 634, 662; polypeptides, 86–90, 132, 133, 509; protein reaction, 101; ichthylepidin, 121; proteoses, 127; cystine and cystinuria, 148, 776; tryptophane, 157, 158; histidine, 160, 161; diaminotrioxydodecanoic acid, 165; glycoproteid, 167; proteoses in the blood, 263, 264; blood serum, 266, 269, 270; blood corpuscles, 275, 277, 304; blood analyses, 328, 334, 338; iron perparations, 340; blood and high altitudes, 341; adrenalin, 376, 380; melanin, 380; cholesterin, 445, 448; gastric contents, 481; digestion, 484, 513; duodenal secretion, 489, 490; absorption and synthesis of proteins, 529, 530; fat, 559; milk, 646, 659, 662, 667; urea formation, 682; nuclein metabolism, 702, 705; amino-acid storage, 721, 722; alcaptonuria, 735–737; urinary sulphur, 752; polypetides in the urine, 763; amino acids in the urine, 756, 827; pyridine, 787; Bence-Jones proteid, 792, 793; tunicin, 839; nutritive value of gelatin, 912; ammonia as protein sparer, 912; water and metabolism 920; alcohol, 921; nin-hydrin, 101; caprine, 144; tyrosine reagent, 154
Abel, J., 379, 621, 683
Abeles, M., 266, 749
Abelmann, M., 532, 539, 540
Abelous, J., 757, 877
Abelsdorf, G., 616
Ach, L., 698
Achalme, 307
Achard, Ch., 265
Acheles, W., 698, 757
Ackermann, D., putrefaction bases, 47; histidine, 82, 159, 160; proline, 154; aporrhegmen, 166; blood corpuscles, 274, 275; meat bases, 578; lysine in the urine, 828
Ackroyd, H., 717
Adam, H., 311
Adamkiewicz, A., 100, 912
Adamson, 17
v. Anrep, R., 287, 723
Adler, O., proteoses, 209; fructose, 217, 814; ochronosis, 550; blood, 797
Adler, R., proteoses, 134; pentoses, 209; fructose, 217; blood, 797
Adrian, C., 730
Adriance, J., 662, 665
Adriance, V., 662, 665
Aducco, V., 675, 757
Agulhon, 50
Albanese, M., 711
Albert, R., 41
Albertoni, P., 415, 502, 533
Albrecht, E., 273
Albro, A., 506
Albu, A., gastric juice, 466; urine poison, 758; mineral metabolism, 758, 769, 899, 901, 902
v. Alder, L., 792
Aldrich, J. B., 846
Alexander, F., 133, 652
v. Alfthan, K., 749
Allard, E., 825
Allaria, G. B., 659
Allen, S., 471
Alleis, R. A., 157, 380
Allihn, F., 656, 811
Allison, F. G., 689
Almagia, M., 410, 583, 706
Almén, A., xanthine, 188; sugar test, 214; meat, 599, 600; food-stuffs, 940
Aloy, J., 877
Alsberg, C., 104, 184
Altmann, R., 179
Amberg, S., 71, 642, 643
Ambronn, H., 839
Amerman, G., 470
Ameseder, Fr., 239, 627, 844
Amiradzibi, S., 575
Amthor, K., 237, 559
Andersen, A. C., 154, 804, 809, 810
Anderson, R. J., 580
Anderson, N., 332
Andersson, J., 376
Andryewskij, P., 874
Anselm, R., 435
Ansiaux, G., 97, 253
Anthon, 213
Appleyard, J. R., 28
Araki, T., blood pigments, 285; nucleic

23; adsorption, 28, 29, 69; dextrin B, 230
Binét, P., 541
Bing, H. J., 245, 331, 385, 398
Bingel, A., 266
Bing, C., 777
Biondi, C., 42
Biot, J. B., 867
Birchard, Fr., 134, 144
Biscaro, G., 653
Bischoff, Th., 879
Bizio, G., 849
Bizio, J., 390
Bizzozero, J., 307, 314
Bjerre, P., 921
Björn-Andersen, H., 769
Blachstein, A., 582
Blanck, F. C., 696
Blankenhorn, E., 606
Blanksma, J., amino-sugar, 167; 219, oxymethylfurfurol, 211, 215, 217; acetone, 825
Bleibtreu, L., 326, 690, 934
Bleibtreu, M., 326, 563, 636
Bléile, A. M., 332
Blendermann, H., 410, 734, 780
Bliss, C. L., 126
Blix, M. G., 326
Bloch, Br., 735, 736
Blondlot, N., 518
Blood, A., 52
Blum, F., haloginized protein, 82; Millons reaction, 99; adrenalin glycosuria, 380
Blum, L., autolysis, 45; protein nitrogen, 77; protogon, 126; alcoptonuria, 735, cystin, 776; tyrosin cleavage, 779, acetone bodies, 818, 819, 822; food value of albumoses, 912
Blumenthal, F., gelatin, 83; indol and skatol, 159; pentoses, 208, 815, 816; nucleoproteins, 383; glycogen, 394; assimilation limit, 534; urine indican, 728; acetone, 818
Boas, J., 488
Bocarius, N., 621
Bocchi, O., 741
Bock, C., 398, 400
Bock, J., 285
Bode, A., 658
Boden, E., 707
Bodländer, G., 921
Bodon, K., 356
Bodong, A., 318, 323
Boedeker, C., 727
Bödtker, E., 680, 759
Boehm, P., 381
Boehm, R., 448, 581, 590, 591
Boehner, R., 154
Boehtlingk, R., 895, 896
Boekelmann, W. A., 826
Boeri, G., 716, 752
Boettger, 214
Bogdanow, E., 586, 596
Bogdanow-Beresowski, 455
Bogen, H., 464
Bogomoloff, Th., 743
Bohland, K., urea nitrogen, 680; urea, 690; uric acid, 701; ammonia, 768; protein need, 934
Bohm, V., 402
Bohmansson, G., 803, 804
Bohr, Chr., blood pigments, 276, 278, 279, 282, 287; egg hatching, 637; gases of blood, 850–853; metabolism in lungs, 858, 869; oxygen tension, 859, 861–863, 867; carbon dioxide tension, 865, 866; specific action of lungs, 864, 867; air-bladder, 867; oxygen capacity, 868
Du Bois-Reymond, E., 592, 602
Du Bois, Reymond, R., 312
Bokorny, T., 212
Bolaffio, C., 630
Boland, G. W., 365
Boldyreff, W., gastric digestion, 481, 511; intestinal juice, 490–493
Boljarski, N., 694
Bolin, J., 873
Boll, F., 615
Bonamartini, G., 96
Bonanni, A., 437, 438, 785
Bondi, J., 642, 643
Bondi, S., lipoproteids, 87; sericin, 122, 123; bile acids, 419, 421, 423; acetoacetic acid, 825
Bondzinski, St., oxyproteic acid, 83; koprosterin, 448; ovalbumin, 633; urine purines, 711; urochrom, 741; oxyproteic acids in urine, 753, 754
Bonnema, A., 646
Bookman, S., 721, 722
Boos, P., 454
Borchardt, L., elastin albumose, 263, 527; sugar formation, 398; fructose uria, 814; acetone, 818, 819, 825
Bordet, J., antienzymes, 64; sensibilizators, 69; blood coagulation, 314, 318, 320
Borissow, P., 462, 717
Borkel, C., 136
Bornstein, K., 595, 918
Boruttau, H., 581
Bosshard, E., 149
Bostock, G., 676
Bottazzi, Ph., freezing point, lowering of blood, 12; blood corpuscles, 304; glycogen, 384, 390, 391; heart muscle, 569; smooth muscle, 602; placenta, 640
Bouchard, Ch., 393, 757, 758
Bouchet, A., 680
Bouchez, 767, 772
Boudet, 264
Boulud, glucuronic acids, 221, 331; pentoses, 264; sugar in blood, 264, 329–332; glycolysis, 333; maltose in urine, 814
Bouma, J., indican, 730; bile pigments, 801, oxybutyric acid, 826

Bourcet, P., 269, 336
Bourquelot, E., 395
Bouveault, L., 144
Brach, H., 839
Bradley, H. C., 500
Brahm, C., 559, 785
Brahm, B., 179, 182, 183, 211
Brand, J., 414, 437, 438
Brandberg, J., 793
Brandenburg, K., 310
Brandl, J., 841
Brasch, W., 144, 534
Brat, H., 209
Brauer, L., 140
Braun, K., 64
Braunstein, A., 408
Brautlecht, C., 78
Bredig, G., colloidal metals 14; surface tension, 26; catalysis, 33–37; assymetric synthesis, 59
Brienl, F., 103
Brenzinger, K., 827
Bretschneider, A., 329, 330
Brewster, J. F., 155
Brieger, L., putrefaction products, 47; neurine, 246; intestinal putrefaction, 514; skatol, 521; neuridine, 606, 612, 628; urine indican, 727, 729; skatoxylsulphuric acid, 732; cystinuria, 827; perspiration, 848
Briggs, C. E., 402
Brigl, P., 179, 183
Brion, A., 774
Brodie, T. G., 256, 400, 509
Brodley, H. C., 72
Brook, F. W., 82
Brooks, Cl., 399
Browinski, J., 266, 741, 754
Brown, A. J., 55, 56, 65
Brown, E. W., 705, 717
Brown, H. T., inverting enzymes, 55; starch hydrolysis, 226, 229, 456, sacharase, 492
Brown, R., 19
Brown, T. Graham, 594
Browne, C. A. (jr.), 647
Brubacher, H., 554, 557
v. Brücke, E., blood coagulation, 316; glycogen, 392; pepsin 467–469, fat emulsion, 511; protein absorption, 525; carbohydrates in urine, 749
Brugsch, Th., bile pigments, 443; pancreas, 532; uric acid, 700, 701; hippuric acid, 721; amino-acids in urine, 757; urine in hunger, 897
Bruhns, G., 193
Brunner, E., 36
Brunner, Th., 665
Bruno, G., 501, 502, 506, 510, 758
Brunton-Blaikie, 572
de Bruyn, Lobry, 19, 201, 220
Bryant, A. P., 936, 938
Buchanan, A., 256
Buchner, E., alcohol fermentation, 41, 205; lactic acid fermentation, 207; sugar test, 213
Buchner, H., 41, 856
Buchtala, H., 113–115, 147, 426
Budde, V., 812
Bülow, K., 95, 227
Bünz, R., 612, 613
Bürger, L., 545
Bürker, K., 314, 315
Bufalini, 339
Bugarszky, St., 271, 326
Buglia, G., 510, 572, 602, 603
Bull, H., 237
v. Bunge, G., serum, 270; blood corpuscles, 304; blood coagulation, 313; blood analysis, 328; iron preparations, 340; blood and high altitude, 341; iron in liver, 387; gastric juice, 485; cartilage, 550; hämatogen, 629, 637; milk, 657, 664, 666–668; hippuric acid, 722, 723; mineral requirement, 900, 901
Bunsen, R., 690
Buntzen, J., 339
Buraczewski, J., 83
Burchard, H., 447
Burckhardt, A. E., 270
Burian, R., purine bases and their enzymes, 193, 195, 371, 373, 572, 594, 702, 703, 713; uric acid formation, 700, 702, 703, 705; uric acid cleavage, 706; histone in urine, 795
Burow, R., 371, 663
Busch, P. W., 349, 350
Butlerow, A., 211, 212
Butterfield, E., 277, 303
Bywaters, H. W., 260, 263

Cade, A., 464
Cahn, A., 476, 615
Camerer, W., milk, 656, 657, 662, 664–666; urine nitrogen, 680; uric acid elimination, 700; purine bases, 715; metabolism, 922, 924
Camerer, W., jr., 847
Cameron, A. T., 78, 109
Camis, M., 279, 592
Cammidge, P. J., 815, 827
Campani, A., 763
Campbell, G., 628, 629, 633, 634
Campbell, J. F., 432–434
Camps, R., 739
Camus, L., pancreatic juice, 496; secretin, 498; vesiculase, 622
Cannon, W. B., 479–484, 524
Cappelli, J., 304, 602
Cappezzuoli, C., 369, 557
Capranica, St., 190, 848
Carbone, D., 613
Carlier, E. W., 313, 347
Carlinfanti, E., 235, 236
Carlini, C., 381
Carlson, A. J., 348, 349, 454
Carlson, C. E., 873

Carnot, Ad., 552, 558
Carvallo, J., 485, 486
Casali, A., 847
Caspari, W., high altitude 341, 888; protein metabolism, 595, 916, milk fat; 669; vegetable diet, 915–916
Castoro, N., 161
Cathcart, E. P., autolysis, 44; glycogen, 396; stomach, 479; gastric digestion, 481; protein absorption, 527; creatine and creatinine, 594, 693; milk sugar, 670; starvation urine, 897
Cavazzani, E., cerebrospinal fluid, 361; glycogen cleavage, 399; absorption, 535, phosphocarnic acid, 578; muscular work, 592; semen, 620
Cernj, C., 838
Cerny, T., 583, 792
Chabbas, J., 617
Chabrié, C., 557
Chandelon, Th., 592
Chaniewski, 563
Charnas, D., 746, 747
Chassevant, A., 706
Chauveau, A., sugar formation, 412; fat formation, 562; muscular work; 592, 597
Cherry, Th., 68
Chigin, P., 462
Chistoni, A., 314, 315
Chittenden, R. H., keratin, 119; elastin, 116, 117; gelatin, 118, 120; albumoses and peptones, 128–131, 137; saliva, 455–458; pepsin, 470, 471; trypsin, 506; tendon mucoid, 545, 551; myosin, 567, 568; neurokeratin, 605, 613, 614; protein requirement, 934
Chodat, R., oxidases, peroxides and peroxidases, 871; oxidation processes, 875; "philothion," 877
Chossat, Th., 892, 896
Christenn, G., 662
Christensen, A., 793
Ciamician, G., 159
Cingolani, M., 699
Citron, H., 766
Clapp, S. H., 89, 107
Clar, C., 701
Clarke, T. W., 287
Claus, R., 408
Clausmann, P., 693, 552, 558
Clemens, Paul, 785, 827
Clemm, C. G., 665
Clerk, B., 265
Cleve, P. T., 422, 423
Cloëtta, M., 290, 292, 293, 753
Cloez, 441
Clopatt, A., 921
Closson, O. E., 400
Cobliner, 332
Cohn, Felix, 485
Cohn, Max, 500
Cohn, Michael, 455
Cohn, R., leucinimid, 145; carbohydrate formation, 410, 411; fate of aromatic substances in the animal organism, 778, 783, 784; furfurol, 784, pyridin, 787
Cohn, Th., 621, 717
Cohnheim, J., 456
Cohnheim, O., lipoid action, 10; proteins, 76, 95; blood and high altitudes, 341, 340; glycolysis; 407, 408; gastric juice, 465; peptic digestion, 481, 484; absorption, 484, 528, 542–543; erepsin, 491, 493; pancreatic juice, 497; connective tissue, 512; peristalsis, 524; digestion work, 930; accommodation of digestive enzymes, 53
Cohnstein, J., 336
Cohnstein, W., 309, 352
Colasanti, G, 591, 593, 698, 748, 749
Cole, S. W., protein reaction, 100; tryptophane, 155–158
Collmann, 849
Comaille, A., 653, 668
Comesatti, G., 380
Comiotti, L., 816
Connstein, W., 265
Conradi, H., 45, 324, 520
Constantinidi, A., 535, 915, 916
Constantino, A., 572
Contejean, Ch., phlorhizin diabetes, 400; gastric juice, 465, 476; pyloric secretion, 477
Copemann, M., 797
Coranda, G., 682
Cordua, H., 301
Corin, G., protein coagulation, 97; fibrinogen, 253; proteins of egg-white, 633
Coronedi, G., 560
Corper, H. J., 372, 449–450, 701, 706
Corvisart, L., 502
Costantino, A., 586, 602, 603, 748
Le Count, E. R., 114
Courant, G., milk, 644, 649, 650, 659
Cousin, H., 242, 248
Couvreur, E., 393
Cramer, C. D., 253
Cramer, E., 122, 848
Cramer, Tr., 531
Cramer, W., absorption, 525, 528, 530; protagon, 606–608; placenta, 640; creatine, 692; hippuric acid, 722; blood coagulation, 320
Cremer, M., glycogen, 58; 332, 390, 393, 395; pentoses, 208; glycolysis, 332; phlorizin-diabetes, 400; sugar formation, 412; fat formation, 562
Cristea, G., 336
Crittenden, A. L., 454
Croftan, A., 334
Croft-Hill, A., 58, 225
Croner, W., 488, 536
Cronheim, W., 488
Croockewitt, J. H., 122
Csökäs, J., 648, 658
v. Csonka, F., 657
Cummis, G. W., 506, 567, 568

Cunningham, R. H., 540
Curtius, Th., 85, 80, 422
Cutler, W. D., 168, 544, 545
Cybulski, N., 379
Czernecki, W., 266, 358
Czerny, Adalb, 307
Czerny, F., 407,
Czerny, V., 485, 525
v. Czyhlarz, E., 873

Daddi, L., 339, 669
Daenhardt, C., 856
Dakin, H. D., autolysis, 44; mandelic acid ester, 62; arginase, 89, 161, 574, 681, 682; protamine, 109, 110; valine, 141; serine, 145; proline, 154; color reactions, 157; lysin, 163; hexon bases, 164; oxalic acid, 716, 773; alcaptonuria, 737; cleavage of fatty acids 774, 781; uramino acids, 786; acetone formers, 822; sugar formation, 412; lactic acid, 583; α-amino acids, 775
Daland, J., 329
van Dam, W., 474, 650
Danilewski, A., plasteines, 58, 135; protein sulphur, 79; retarding substances, 487, 492; muscle protein, 566–569; milk globules, 646
Danilewsky, B., 885
Danilewsky, W., 241
Dareste, C., 620, 628
Darmstädter, E., 826
Darmstädter, J., 846
Darmstädter, L., 448
Dastre, A., fibrinogen, 252; fibrinolysis, 255; glycogen, 307, 346; blood coagulation, 314; liver, 383, 384, 388; glycogen, 395, 397, 399; bile, 414, 434, 435, 511; enterokinase, 496, 497; fat absorption, 538
Dantzenberg, P. J. W., 783
Danwe, F., 49
Davidoff, W., 75
Day, H., 480
Dean, A. L., 228, 529
Decaisne, E., 667
Deetjen, H., 308, 314, 315
Dehn, W. M., 760
Dekhuysen, C., 13
Delezenne, C., enzyme retardation, 65; papain, 65; blood coagulation, 250, 313, 318, 320, 324, 325, 351; intestinal juice, 490–492, enterokinase, and pancreatic juice, 493, 495–497, 509; secretin, 498
Delfino, A., 640
Demant, B., 491
Demoor, J., 453
Denigès, G., tyrosin, 154; indol and skatol reactions, 158, 159; inosite, 580; homogentisic acid, 739
Denis, P. S., 257
Denis, W., tyrosin, 154; urea in blood, 333, 336; hypophysis, 592; creatine, 692, 696; uric acid, 708, 711, 334; urine sulphur, 752
Denk, W., 336
Dennemark, L., 658
Derrien, E., glucose, 215; blood pigments, 281, 285, oxymethylfurfurol, 419; Bence-Jones, protein, 792
Desgrez, A., 393
Dencher, P., 532, 539, 540
Devilard, P., 360
Devoto, L., 792
Dewitz, J., 843
Diakanow, C., 243–245
Diamare, V., 494
Dick, M., 702
Diels, O., 445, 446, 448
Diesselhorst, G., 848
Dietrich, M., 104, 652
Dietschy, R., 792
Dietz, W., 56, 60
Dietze, Alb., 506
Dillner, H., 633
Dimitz, L., 248, 614
Disqué, L., 740, 742, 744
Ditthorn, Fr., 167, 173, 219
Dittrich, P., 285
Ditz, H., 726
Döblin, A., 329, 333
Dörpinghaus, Th., carbohydrates in protein substances, 84; protein hydrolysis, 113, 141, 142, 252
Dohm, M., 406
Dombrowsky, St., urinary pigments, 740, 741; oxyproteic acids in urine, 753, 754; urinary bases, 757
de Domenicis, A., 287
de Dominicis, N., 404
Donath, J., 360
Donné, A., 799
Dony-Henault, O., 873
Donzé, G., 680
Dorée, Ch., 448
Dormann, E., 298
Dorner, G., 574
Douglas, C. G., 286
Douglas, Gordon C., 864
Doyon, M., fibrinogen, 252, 253, 335; serum lipase, 265; blood coagulation, 324, 325; glycolysis, 332; bile, 415, 416, 434, 439
Dragendorff, D., 800
Drechsel, E., proteins, 76, 79, 94, 123; diamino-acetic acid, 163; lysin, 163, 164; purin bases, 188; thyroid gland, 375; jecorin, 385; urea formation, 681, 683; carbamic acid, 683; silicic acid ester, 838
Dreser, H., 13, 71
Dreyfus, G. L., 465
Droop-Richmond, H., 646
Drosdoff, W., 335
Dubelir, D., 920
Ducceschi, V., 475, 569
Duclaux, E., 97, 647

Ducleau, E., 55
Dudley, H. W., 583, 775
Düring, Fr., 554
Dufau, E., 808
Dufourt, 415, 416, 439, 592
Duggan, C. W., 97
Dull, G., 229
Dumas, J. A., 684
Dunham, E., 180, 239, 673
Dunlop, J. C., 595, 715

Ebbeke, U., 757
Ebstein, E., 114, 208, 209
Ebstein, W., 457, 727, 831
Eckhard, C., 452
Edelstein, E., 43
Edelstein, F., 657, 662
Edic, E., 246, 402
Edkins, J. S., 463, 477, 509
ver Eecke, A., 925
Ehrenfeld, R., proteins, 83; leucine, 143; tyrosin, 153
Ehenreich, M., 49, 505
Ehrenthal, W., 521
Ehrlich, F., amino-acids, 143, 144, 153, 206; fermentation, 157; fusel oil, 206
Ehrlich, P., side chain theory, 67, 68; amboceptors, 69; dimethylaminobenzaldehyde, 159, 219, 746, 827; bilirubin, 431; urine test, 826
Ehrmann, R., 405
Ehrström, R., histone, 108; phosphate, 761, 762, 796; albumoseuria, 791
Eichholz, A., 260, 633, 743
Eichhorst, H., 525
Einhorn, M., 159
Ekbom, A., 426
Ekehorn, G., 760
van Ekenstein, A., amino-sugar, 167, 219; carbohydrates, 201, oxymethylfurfurol, 211, 215, 217; acetone, 825
Ekholm, K., 936
Elias, H., 609
Ellenberger, W., 479, 480, 658, 659
Ellinger, A., isoserin, 145; tryptophan, 155, 156; arginin, 163; blood coagulation, 324; lymph formation, 351, 352; pancreatic secretion, 500; urine indican, 728, 730; tri-indylmethane pigments, 734; kynurenic acid, 739; oxyphenyllactic acid, 780; food value of albumoses, 912
Ellmer, A., 237
Ely, J., 455, 457
Embden, G., cystein and cystine, 79, 147, 149; serine, 145; glycolysis, 333, 408; carbohydrate formation. 410; liver blood perfusion, 529, 530, 775, 786; lactic acid, 583, 584, 333; glycocoll in urine, 756; acetone bodies, 819 822, 825, 826
Embden, H., 735
Emerson, R. L., 507
Emich, Fr., 517
Emmerling, A., 113
Emmerling, O., 58, 59, 225
Emsmann, Otto, 463
Engel, 662
Engel, H., lipases, 57, 476; lactic acid formation, 585; acetone bodies, 818, 821
Engel, St., 658, 663
Engeland, R., agmantin, 162; aporrhegmen, 166; carnitin, 577; methyl-guanidin, 698; urine bases, 757, 826, 827
Engelmann, G. J., 595
Engler, C., 871, 875
Eppinger, H., 375, 406, 676, 799
Eppinger, P., 290, 292, 293, 354
Epstein, A., 721, 722
Erben, Fr., oxystearic acid, 233; leucocytes, 307, 342; blood analysis, 342; chyle fat, 346; urine nitrogen, 681, urein, 691
Erdélyi, A., 482
Erikson, A., 50, 63
Erlandsen, A., phosphatides, 242, 243, 245, 246, 586; cuorin, 248, 249; phlorhizindiabetes, 400
Erlanger, J., 541
Erlenmeyer, E., 142, 152
Erlenmeyer, E. (jr.) 145, 148, 152
d'Errico, G., 391
Esbach, G., 793
Escher, Heins, H., 623, 631
Estor, A., 867
Etard, A., 578
Etti, C., 642
Ettinger, J., 43
Eulenburg, 398
v. Euler, H., activators, 52; enzymes, 53. 56, 70; phosphoric acid ester, 205; erepsin, 493; oxidation processes, 873
Eves, F., 457
Ewald, Aug., proteins, 112, 119; hæmatoidin, 301; digestion, 508; visual purple, 615; corpora lutea, 623
Ewald, C. A., 857, 928
Ewins, A. I., 380
Eykman, C., 326, 929, 932
Eymonnet, 757

Fabian, E., 396
Fahr, G., 587
Fajans, K., 35
Falck, 910
Falk, Edm, 642
Falk, Ernst, 924, 925
Falk, Fr., 248, 613
Falloise, A., bile, 416; gastric lipase. 476; intestinal enzyme; 492, 493; chloralsecretin, 499; blood gas tension. 861, 866
Falta, W., thyroid gland, 375; diabetes 405, 406, 409, 412; alcaptonuria, 735. 736; protein metabolism, 882, 883, 907
Fano, G., 251
Farkas, K., 638

Farmer, Ch., 688
Farwik, B., 599
Fasal, H., 106, 107, 158, 652
Faust, E., sepsine, 47; gelatin, 118; poisons in the secretion of the skin and salivary glands, 847
Favre, P. A., 848
Fawitzki, A., 685
Fedeli, C., 686
Feder, L., 682
Fehling, H., 214, 808
Fehrsen, A., 338
Feigin, P., 721
Feinshmidt, J., 408
v. Fenyvessy, Bela, 751, 785
Fermi, Cl., 255, 486, 505
Fernet, E., 855
Ferry, E., 904
Fick, A., 596
Fiessinger, N., 307, 364
Filchne, W., 440
Filhol, 670
De Filippi, F., 397, 534, 757
Fine, M. S., 692
Fingerling, G., 667
Fink, H., 295
Finkler, D., 41
Fischer, Ch., 140
Fischer, Emil, enzymes, 58, 59; specificity of enzymes action, 62, 65; amino-acids, 85, 140–145, 148, 152–155; polypeptides, 86–89, 124, 132, 133, 136; protein hydrolysis, 113, 119, 120, 132, 133, 136, 507; ornithin, 162; lysin, 163; diaminotrioxydodecanoic acid, 165; purin bases, 186, 188–191; pyrimidine bases, 194, 195; carbohydrates, 197–202, 211, 212, 215, 216; glucosamine, 218; glucuronic acid, 220; isomaltose, 225; lactose fermentation, 654; uric acid, 698; urine purines, 711; conjugated glucuronic acids, 751
Fischer, Hans, blood pigment derivatives, 295-297, 295; bile acids, 425, 427; bilirubin, hydrobilirubin, urobilinogen and urobilin, 429, 744, 746, 747, 429; koprosterin, 448; urobilinoids, 743; bilirubic acid, 429
Fischer, H. W., 587
Fischer, Martin, 400
Fischler, E., 686
Fischler, M., 434, 744
Fisher, H. L., 694
Fiske, P. S., 36, 38
Fitzgerald, 476
Flack, M., 374
Flächer, Fr., 379
Flamand, Cl., 152, 153, 734
Flanders, Fr., 723
Flatow, L., 738, 739, 779, 780
Flatow, R., 711
Fleckseder, R., 455
Fleig, C., 416, 498, 499
Fleischer, R., 402
Fleischl, E., 303, 441
Fleischmann, W., 647
Fleischer, M. S., 320
Fleitmann, 79
Fletcher, W. M., 459, 585, 593
Flint, A., 448, 488
Floresco, N., 384, 388, 434
Flückiger, M., 749, 750
Foà C., 455, 645, 659
Folin, O., cystine, 149; tyrosine test, 154; blood alkalinity, 309; urea, 333, 335; urine acidity, 677; nitrogen determination, 688; urea determination, 688–690; urein, 691; methylurea, 691; creatinine and creatine, 691–693, 696, 697; uric acid, 700, 708, 711, 334; hippuric acid, 723; urinary sulphur, 752, 766; ammonia, 768, 769; acetone, 825; protein metabolism, 910
Fordos, M., 365
La Forge, F., 179, 180, 185
Forrest, J. R., 553
Forschbach, J., 396
Forssner, G., 756, 819, 820
Forster, J., mineral metabolism, 72, 899; transfusion, 344; water and metabolism, 920; metabolism of sucklings, 925; diets, 932, 939
Fosse, R., 681
Foster, M. L., 159
Foster, N. B., 694
Fränckel, P., 465
Fränkel, A., 685, 860
Fränkel, Sigm., proteins, 79, 102; peptones, 131; thiolactic acid, 151; histidin, 160; albumin, 219; cephalin, 248; thyroids, 375; adrenalin, 380; glycogen, 396; gastric juice, 476; chondrosin, 548; brain phosphatides, 605, 609; brain analyses, 613, 614; neottin, 630, kidney phosphatides, 673; homogentisic acid, 738; chitin, 839; protein cleavage, 925
Fränkel, W., 34
Framm, F., 120, 213, 332
Franchimont, A. P., 839
Frank, E., 265, 329–332
Frank, Fr., 702, 706
Frank, O., 535–537, 591. 910
Frankland, E., 885
Franz, Fr., 251
Frauenberger, Fr., 547
Frazer, J. C. W., 3
Frédéricq, L., proteincoagulation 97; serumglobulin, 260; hæmocyanin, 203, 304; blood coagulation, 313· blood gases, 852, 861, 862, 864, 866
Frehn, A., 663
Frémy, E., 603, 636
Frenkel-Heiden, 360
Frenzel, J., glycogen, 391, 393; work and fat destruction, 596, 598; meat, 600; calories and nitrogen, 892
Frerichs, F. Th., synovia, 362, human

bile, 437; saliva, 458; uric acid decomposition, 705
Freudberg, A., 309
Freund, E., serumglobulins, 259; albumoses in blood, 263; hæmatinogen, 299; blood coagulation, 313, 320; glycogen, 395; digestion blood, 530; chlorine determination, 759; lungs, 870; starvation metabolism, 987
Freund, O., 897
Freundlich, H., 22; surface tension, 28; adsorption, 29
Frey, W., 756
Freytag, Fr., cerebrosides, 364, 607, 610, protagon, 606, 607, 610
Fridericia, L. S., 597
Friedenthal, H., 467, 525
Friedjung, J. K., 664
Friedländer, G., 525
Frienländer, P., 843
Friedmann, E., protein sulphur, 79; thiolactic acid, 113, 151; albumoses, 134; isoleucine, 144; cystine, 148; cysteinic acid and taurine, 148, 151, 442; adrenalin, 379; demolition of fatty acids, 774, 776, 781; of aromatic substances, 779–781, 786; furfurol, 784; acetone former, 820, 822
Friend, W. M., 274, 356
Fries, H., 593
Fröhlich, 379
Fromherz, K., 735, 736, 780
Fromholdt, G., 743, 744
Fromm, E., 785
Fromme, A., 57, 476
Frommer, V., 823
Frouin, A., thyroid gland, 377; gastric juice, 463, 464; intestinal juice, 490–492; pancreatic juice, 496
Frugoni, C., 405
Fubini, S., 849
Fuchs, D., 114, 154
Fürbringer, P., 715, 791
v. Fürth, O., peroxyproteic acids, 83; xanthoprotein, 83; nucleic acids, 184; cholin, 247; iodothyrin, 376; suprarenin, 379; adrenalinglucosuria, 405; cholic acid, 424; secretin, 498; bile and fat splitting, 502; muscles, 566–572, 589, 590; camosin, 575; smooth muscles, 602; chitosin, 839; melanins, 841–843; tyrosinase, 843, peroxydase, 873
Fuld, E., rennin action, 57, 474, 650; fibrin formation, 256, 318, 319, 324; pepsin determination, 470; womans' milk, 660, 662
Fuller, J. G., 902
Funk, C., amino-acid combination, 87, 88; glutamic acid, 146; urinary sulphur, 752; sugar determination, 809; vitamine, 905
v. Funke, 595
Gabriel, S., cystine, 148; bones, 552; teeth, 558; ovalbumin, 633; food-value of aspargin, 912
Gache, J., 496
Gaglio, G., 334, 582, 773
Galdi, F., 354
Galeotti, G., 96, 706
Galimard, J., 113
Galli, P., 267
Gallois, 580
Gamgee, A., nucleoproteins, 175; nucleic acids, 182; blood pigments, 280; intestinal juice, 490; protagon, 606, 607; pseudocerebrin, 611
Gammeltoft, S. A., 689, 690, 767
Ganassini, D., 455, 456, 709
Gansser, E., 278
Gardner, J. A., 448
Garrod, A. E., hæmatoporphyrin, 295, 299, 797; alcaptonuria, 738; homogentisic acid, 738, 739; urochrome, 740, 741; urobilin, 743, 745; uroerythrin, 748; cystinuria, 827
Gaskell, J. F., 828
Gassmann, Th., 552, 557, 558
Gatin-Gruzewska, Z., glycogen, 19, 20, 390, 391; seralbumin, 261
Gaube, T., 848
Gaule, J., 340
Gaunt, 41
Gautier, A., ptomaines, 47; arsenic, 72, 269, 336, 368, 373, 838; glycogen, 392; fat formation, 562; muscles, 578; protein of hen egg, 633, 634; xanthocreatininine, 698; fluorine, 552, 558
Gautier, Cl., 252, 253, 654, 744
Gawinski, W., 756
v. Gebhardt, F., 591, 909
Geelmuyden, H. C., sugar in urine, 814, 816; acetone bodies, 819, 820, 822, 826
Geiger, W., 145
Geissler, 791
Generali, F., 374
Gengou, O., 64, 314, 318, 320
v. Genser, 665
Gentzen, M., 728
Geoghegan, E. G., 609, 610, 614
Gephart, F., 245, 680, 689, 769
Geppert, J., blood alkalinity, 309; respiration, 860, 869, 890; alcohol, 921
Gerard, E., 446, 699
Gerber, N., 662
Gerhardt, C., 824
Gerhardt, D., 744, 745
Gerhartz, H., 870
Gerngross, O., 195
Gertner, W., 416
Gessard, C., 843
Gewin, J. W. A., 475
Geyer, J., 806
Geyger, A., 735
Giacosa, P., mucins, 167; blood pigments, 302; frog egg, 636; iron in urine, 770; aromatic substances, 779

Giaja, J., 71, 231
Gibson, R., 103
Giertz, H., 104, 177
Gies, W. J., elastin, 116–118; hexone bases, 164; mucin substances, 168, 169, 472, 544, 545, 551; lymph, 349–351; pancreatic juice, 500; ligaments and tendons, 545; bones, 551; protagon, 606, 608; phrenosin, 611; urein, 691
Gigon, A., polypeptides, 65; diabetes, 405, 409; amino-acid supply, 721, 722; amino-acids in urine, 756; basal-requirement, 898, 931; metabolism, 929–931
Gilbert, 531, 563
Gill, F. W., 689, 752
Gilson, E., 242, 245, 839
Ginsberg, S., 534
Ginsberg, W., 754, 756
Githens, Th., St., 270
Giunti, L., 773
Gizelt, A., 498
Gjaldbäk, I. K., 58, 135, 165
Glaessner, K., pseudopepsin, 466, 489, 490; erepsin, 493; pancreatic juice, 500, 509
Gleiss, W., 593
Glendinning, T. A., 55
Gley, E., iodine in blood, 269; blood coagulation, 324; lymphagogues, 351; thyroidea, 374; pancreatic juice, 496, 498; heart muscle; 599, vesiculase, 622
Glikin, W., fat, 233; lecithin, 241, 244, 245, 663; liver nitrogen, 387; cholesterin, 445; milk, 663
Glund, W., 87
Gmelin, L., 432, 458
Gmelin, W., 465
Gogitidse, S., 669
Goldmann, E., cystine and cystinuria, 149, 827, 828; iodothyrin, 376
Goldmann, F., 813
Goldschmidt, C., 687
Goldschmidt, F., 133
Goldschmidt, H., 36, 456
Golodetz, L., 844
Gompel, M., 65
Gonnermann, M., 140, 508
Goodbody, W., 515
Goodwin, R., 130
Gorodecki, 443
Gortner, R. A., 841, 842
v. Gorup-Besanez, E. F., 356, 437, 838
Gossmann, H., 495
Gosio, B., 582
Goto, M., 110, 111, 709
Gottlieb, R., urea, 333; bile, 435; creatine and creatinine, 573, 692, 698; urine purines, 711; oxyproteic acid, 754; iron in urine, 770
Gouban, F., 366, 367
Gourlay, F., 373
de Graaf, C. J. H., 792, 793
Grabowski, J., 297
Graebe, C., 524
Graffenberger, L., 554
Grafe, E., 54, 912, 919
Graham, D., 919
Graham, Th., 13, 14, 18, 31, 96
Granström, E., 718, 769
Grawitz, E., 340
Grebe, F., 396
Green, E. H., 121
Green, J. R., 255, 316
Greer, James Richard, 348, 349
Gregersen, J. P., 903
Gregor, A., 698
Gregor, G., 766
Gréhant, N., 333, 335, 684, 773
Griesbach, W., 333
Griffiths, A. B., 614, 758
Grimaux, E., 85, 86, 323
Grimbert, L., 677, 746, 747
Grimm, F., 744
Grimmer, W., 482, 490, 874
Grindley, H. S., 689, 752, 936
Griswold, W., 457
Grober, J., 467
Gröber, A., 732
Grohé, B., 485
Groll, S., 339
Grosjean, A., 324
Gross, A., 358, 629
Gross, O., pepsin determination, 469; trypsin determination, 506; lens, 619; alkaline earths in urine, 769
Gross, W., 463, 780, 818
Grossenbacher, H., 372
Grosser, P., 733
Grossmann, H., 808
Grouven, H., 599
Grube, K., 396, 400
Gruber, D., 229
Gruber, M., 881, 920
Grübler, G., 94
Grünbaum, D., 642
Gründler, J., 777
Grünhagen, A., 361
Grützner, B., 790
Grützner, P., pepsin determination, 469; gastric contents, 479, 480; Brunner's glands, 489, 490; pancreatic diastase, 501
Grund, G., 208, 209, 389, 919
Grutterink, A., 792, 793
Gryns, G., 7
Gscheidlen, R., rhodan, 455, 456, 751; lactic acid, 582; urea, 684
Gubler, A., 348, 349
Gudzent, F., 707, 708
Gümbel, Th., 77
Günther, G., 622
Gürber, A., ion permeability, 8, 310; serumalbumin, 261, 263; serum, 267; bile, 440; amniotic fluid, 642
Guerin, G., 758

Guest, H. H., 106, 107
Guggenheim, M., 50, 380
Guigan, H., Mc, 399
Guillemonat, A., 371, 387
Guinochet, E., 358
Guldberg, C. M., 32
Gulewitsch, W., arginine, 161, 162; thymin, 195; choline, 247; trypsin, 508; meat extractive, bases, 575–578
Gullbring, A., 421
Gumilewski, 491
Gumlich, E., 680, 685, 761
Gundermann, K., 82
Gunning, J. W., 823
Gusserow, A., 716, 717
Guth, F., 233
Guyenot, E., 457
Gyergyai, A., 912

de Haan, J., 306
Haas, E., 528, 910
Haberlandt, L., 265
Habermann, J., proteins, 83; amino-acids, 143, 146, 153
Hämäläinen, J., glucuronic acid conjugation, 785; sulphur elimination, 882, 883; protein catabolism, 907
Händel, M., 549
Hänsel, E., 487, 663
Häser, 771
Häusermann, E., 340
Hafner, A., 233
Hagemann, O., skin breathing, 849; blood gases, 851; metabolism, 926, 927, 929
Hagen, W., 193
Hahn, M., fermentation, 41; casein digestion, 651, urea formation, 683, 684
Haig, A., 701
Haiser, F., inosinic acid, 178–180; 182, 183; carnine, 577
Haldane, J., blood pigments, 285, 286, 302; amount of blood, 343; oxygen tension, 862, 863
Hall, W., purine bases, 193, 520, 572; iron absorption, 340; amino-acids in urine, 757
Hallauer, B., 440
Halle, W. L., 380
v. Hallervorden, E., 682, 685, 767
Halliburton, W. D., protein coagulation, 97; dextrins, 234, choline; 246; fibrin ferment, 256; serum albumin, 261; blood serum, 270; stroma of blood corpuscles, 274; tetronerythrin, 304, 843; leucocytes, 306; blood coagulation, 323; pericardial fluid, 356; cerebro-spinal fluid, 360; liver, 382; glycogen, 391; pancreatic renin, 509; myxœdema, 545; bone marrow, 553; muscles, 566–570; brain proteins, 604, 605; diseases of the nervous system, 614; kidneys, 673
Halpern, H., 372
Halsey, J. T., 152
Ham, C. E., 301
Hamburger, C., 265, 456
Hamburger, E. W., 435
Hamburger, H. J., blood corpuscles, 6–8, 273, 304, 305, 311; blood alkalinity, 269; blood serum, 271; phagocytosis, 306; blood alkalinity, 309, 310; lymph formation, 350, 351; ascitic fluid, 359; intestinal juice, 490, 491; enterokinase, 496; absorption, 542–543
Hammarsten, O., rennin action, 54, 63, 473, 474, 475, 650; nucleoalbumin, 105; mucin substances, 168–169; helicoproteid, 173; nucleoproteins, 208, 383, 494, 495; fibrinogen, fibrin and blood coagulation, 253, 254, 257, 263, 316; fibrin globulin, 258; globulins, 259–261, 263, serum-albumin, 262; blood plasma and serum, 267–270, hæmatoporphyrin, 299, 797, 798; gases of lymph, 346, 856; transudates, 354, 356, 357, 359; synovia, 362; human bile, 414, 437, 438; bile of various animals, 417, 427, 439; bile acids 417, 420–427, 438; bile pigments, 267, 432, 434, 801; phosphatides, 435, 439; saliva, 457; pepsin, 466, 467; trypsin, 503; pseudomucin, 625, 626; perch eggs, 630, 636; casein, 649; lactoprotein, 653; urea in bile, 679; protein in urine, 793; sugar determination in urine, 806
Hammerbacker, F., 458
Hammerl, H., 521
Hammerschlag, A., 308, 309
Hammerschlag, G., 637
Handovsky, H., 18
Henriot, M., lipases, 59, 265; diabetes, 404; respiration quotient, 563; respiration, 869
Hansen, C., lecithin, 242; protein synthesis, 529; fatty tissue, 559; fat of yolk, 630; fat of milk, 669; food value of albumoses, 912; asparagin, 912
Hansen, W., 233
Hanssen, O., amyloid, 171, 172, 173, 547
Harden, A., co-enzyme, 52, 205; phosphoric acid ester, 60, 205; dioxyacetone, 205; glycolysis, 407, 583
v. Hardt-Stremayr, 77
Hardy, W. B., colloids, 15, 20, 21, 25, 26, 30; ovalbumin, 634
Hari, P., 54
Harkink, I., 594, 693
Harley, V., sugar of blood, 398; intestinal putrefaction, 515; pancreas, 532, 539, 540; large intestines, 541, urinary pigments, 740, 744
Harms, H., 552
Harnack, E., ash-free protein, 95; iodospongin, 122; blood pigments, 286,

287; hydramnion, 642; oxalic acid poisoning, 728, 729; sulphur in urine, 752; demolition of halogen substituted methane, 777; galic and tannic acid, 785
Harries, C., 83
Harris, J. F., 77, 179, 185
Hart, A. S., 116, 117
Hart, E., protein nitrogen, 77; albumoses, 134; histidine, 160; hexone bases, 164;
Hart, E. B., 902
Hartley, P., 384, 385
Hartogh, 412
Hartung, C., 636
Hartwell, J. A., 130
Hasebroek, K., lecithin, 245, 515; pericardial fluid, 356; digestion products, 472
Haskins, H. D., 680, 683, 691
Haslam, H. C., salting out of proteins, 95, 250; albumoses, 139
Hasselbalch, K. A., reaction of blood, 75, 310; methæmoglobin, 283, 284; egg-hatching, 637; sugar determination, 814; oxygen intake, 859; oxygen tension, 867
Hatcher, R. A., 397
Hauff, 665
Hausmann, W., nitrogen in proteins, 77, 119; hæmatoporphyrin, 295; koprosterin, 448; cholesterin, 449–450
Hawk, P. B., keratin, 112; bones, 551; Eck's fistula, 683; sugar determination, 804; hair, 838; metabolism, 892, 897, 920
Hay, M., 358
Haycroft, J. B., protein coagulation, 97; blood coagulation, 251, 313; diabetes, 403; biliverdin, 433
Hayem, G., 307, 342
Heckel, F., 141
Hedenius, 116, 434
Hedin, S. G., blood corpuscles, 7, 8; autolysis, 42–44; adsorption, 49, 50; enzymes, 54–57; retardation of enzyme action, 63–65; rennin-antirennin binding, 68; elastin, 116; histidine, 159, 160; arginine, 161; lysine, 163; blood, 326; hæmatocrit, 326; lienases, 371; rennin zymogen, 473; rennin enzyme, 474; muscle protease, 571
Hédon, E., pancreas diabetes, 406; absorption of sugar, 533; of fat, 539, 540
Heffter, A., liver, 385; muscle, 565, 589; lactic acid, 593; hyposulphite in urine, 753; foreign substances in urine, 773; hippuric acid synthesis, 783; reductions, 877
Heger, P., 381
Heidenhain, M., 101
Heidenhain, R., lymphagogues and lymph, 11, 345, 349–351; transudates, 353; bile, 414, 415; saliva, 453, 459; stomach, 461, 476, 477; pyloric secretion, 477; pancreas and its secretion, 494, 495, 497, 503; absorption, 528, 534; smooth muscles, 602
Heilner, E., protein assimilation, 525; metabolism, 920, 929; specific dynamic action, 930
Heinemann, H. N., 597
Heinsheimer, Fr., 476
Heintz, W., 235, 582
Heiss, E., 555
Heitzmann, C., 555
Heckma, E., intestinal juice, 490–493, enterokinase, 496, 497
Hele, T. Sh., 738, 828
Heller, Fl., protein test, 99, 789; uroxanthin, 727; urinary pigments, 740; blood test, 796; urinary calculi, 834–835
Hellgren, W., 931
Hellwig, 602
Helm, 461
Helme, W., 882, 883, 907
Hetper, J., 292
Hemmeter, J. C., 463, 464, 519
Hempel, E., 894
Henderson, L. J., 309, 675, 677
Henderson, Y., 77, 402, 529
Henkel, Th., 647
Henneberg, W., 510, 918
Henninger, A., 137
Hénogque, A., 303
Henri, V., cataphoresis, 50, 51; enzymes, 56; white of egg, 65; saccharase, 65; diastase, 71
Henriques, R., 239
Henriques, v., plastein, 58, 135; formol titration, 165; lecithin, 242; lecithin sugar, 331, 332; protein synthesis, 529; fat tissue, 559; fat of yolk, 630; fat of milk, 669; urea determination, 689, 690; hippuric acid, 723; urine nitrogen, 756; ammonia, 768; gases of blood, 851; metabolism in lungs, 858; food value, albumoses, 912; asparagin, 912
Hensel, Marie, 726
Hensen, V., 349, 856
Henze, M., gorgonin and iodogorgonic acid, 123, 146; protein of octopodes, 175; hæmocyanin, 303, 304; blood corpuscles in ascitic fluid, 304; liver, 388; spongosterin, 448; muscles, 603
Heptner, F. K., 438
l'Héritier, 665
Herlaut, L., 185
Herlitzka, A., 455
Hermann, E., 72
Hermann, L., blood in starvation, 339; formation of feces, 521; muscular work, 592; allantoin, 716, 717
Heron, J., 229, 456, 492
Herrmann, A., 507, 700, 701
Hermann, Edm., 336
Herry, A., 325

Herter, C. A., indol and skatol, 159, 732; urorosein, 732, 733
Herter, E., saliva, 458; ethereal sulphuric acids, 724, 726, 784; oxybenzoic acids, 783; oxygen tension, 861
Herth, R., 137
Hervierux, Ch., indol and indican, 266, 728–732; skatol red and urorosein, 733; uroerythrin, 748; glucuronic acids, 817
v. Herwerden, M., 651
Herzen, A., spleen and digestion, 372; gastric juice secretion, 462; pancreatic juice, 496, 498
Herzfeld, A., 203, 224
Herzfeld, E., 267, 433
Herzog, R. O., enzymes, 41; histidin, 160; lactic acid, 582, 616; oxydase reactions, 875
Hess, K., 297
Hess, L., 43
Heubner, O., 258, 569
Heuss, E., 847
Hewlett, A. W., 541
Hewlett, R. T., 97
Hewson, W., 6, 313
Heyl, F. W., 185
Heymann, F., 626
Heynsius, A., 128, 432–434
Hiestand, O., 245
Hilbert, P., 779
Hildebrandt, H., oxalic acid, 716, 773; amino-benzoic acids, 783; toluols, 783; glucuronic acid conjugation, 785, 786
Hildebrandt, P., antiemulsin, 64; mammary glands, 644, 668; glucuronic acids, conjugation, 750
Hildebrandt, W., 267, 744
Hildesheim, 932
Hildesheimer, A., 726
Hilger, 171
Hilger, A., 579
Hill, A. V., 277, 278
Hiller, E., 554
Hirsch, Rahel., glycolysis, 408; hippuric acid, 721; amino-acids in urine, 756
Hirsch, Paul, 722, 912
Hirschberg, A., 307
Hirschfeld, E., 485
Hirschfeld, F., work and nitrogen elimination, 595; uric acid, 700; acetone bodies, 819; protein catabolism, 903, 915, 934; daily ration, 939
Herschfeld, H., 791
Hirschl, J. A., 806
Hirschler, A., 517, 518, 519, 724
Hirschstein, L., 700, 756
His, W., 550
His, W. (jr), 193, 707, 787
Hlasiwetz, H., 146
Hochhaus, H., 340
Höber, R., osmotic pressure, 13, 15; precipitation properties of ions, 25; alkalinity of blood, 310, 311; absorption, 542; permeability, 588; urine acidity, 677
Höne, J., 800
Hoernes, Ph., 78
Hörth, F., 582
v. Hoesslin, H., 909–911, 917
Höyrup, M., 161
Hofbauer, L., 456
van't Hoff, J., osmotic pressure, 2, 3; catalysis, 33, 35; glucosides, 59
Hoffman, Ch., 152
Hoffmann, A., 722
Hoffmann, F. A., transudates, 354, 357, 361, 362; sugar in blood, 398; glucoscuria, 400
Hoffmann, J., 675
Hoffmann, P., 770
Hofmann, tyrosin test, 153
Hofmann, Fr., 559–561
v. Hofmann, Karl, 621
Hofmann, K. B., 116, 124
Hofmeister, F., gelatin, 31; cell enzymes, 44; protein nitrogen, 77, 82; amino-acids, binding of 85; removal of proteins, 102; collagen and gelatin, 118, 120; albumoses and peptones, 133, 136, 139; grouping of proteins, 159; serum-globulins, 259, 261; pus, 364; actions of stomach, 479, 480; protein absorption, 527, 528; assimilation limit, 533; blood serum and earthy phosphates, 557; ovalbumin and protein crystallization, 633, 634; urea formation, 684; creatinine, 694; protein in urine, 793; lactose in urine, 815
Hofmeister, V., 480, 510
Hohlweg, H., 267, 740, 741
Holde, D., 233
Hollinger, A., 328
Holmgren, E. S., 452, 806
Holmgren, Fr., 852, 866
Holmgren, I., 489, 566, 569
Holobut, Th., 696
v. Holst, G., 169, 354, 362
Holsti, O., 903
Honoré, Ch., 526
v. Hoogenhuyze, C. J., creatine and creatinine, 573, 594, 691–694
Hooker, D., 351
Hooper, C. W., 442
Hopkins, F. G., halogen proteins, 82; protein reaction, 100; tryptophan, 155–158; protein crystallization, 263, 633, 634; lactic acid formation, 533; uric acid, 699, 710, 711, 843; urobilin, 743, 746; Bence-Jones protein, 792; butterflies, 843
Hoppe-Seyler, F., oxydation, 41; ovovitellin, 105; collagen, 118; proteins, 167; nucleins, 175; xanthin, 189; lecithin, 243–245; blood plasma, 269; blood corpuscles, 274, 304, blood pigments, 275–278, 281, 283, 286, 287–290, 293–302; urobilinoids, 295, 743;

glycogen, 307, 390; blood analysis, 328; chyle, 347; pericardial fluid, 356; pus, 363-365; strumacystica, 374; bile, 435, 436, 440; excretin, 523; cartilage, 549, 550; bones and teeth, 553, 558; lactic acids, 583, 585, 593, 748; retina, 615; ovovitellin, 628; milk, 647, 648, 656; bile acids in urine, 800; inosite, 817; chitin, 839; sebum, 844; skin breathing, 849; respiration apparatus, 868; oxydation, 871
Hoppe-Seyler, G., blood pigment determination, 302; phenol elimination, 725; indoxyl, 728–732, urobilin, 744, 745, 747
Hopwood, A., 87
Horbaczewski, J., keratin, 112; elastin, 116, 117; purine bases, 188, 189; uric acid, 373, 698, 700–702; urostealith, 834, 835; metabolism, 904
Hornborg, A. J., 464, 465
Houre, R. M., 251
Horodynski, W., 334, 336
Horowitz, L. M., 520
Horton, E., 59
Hoshiai, Z., 787
Hougardy, A., 261
Howe, P. E., 892
Howel, W. H., 257, 266, 319, 322
Hryntschak, Th., 723
Huber, A., 251, 255
Hudson, C. S., 55, 57, 199
v. Hübl, 236, 238
Huebner, R., 159
Hüfner, G., leucin, 142; blood pigments, 277–279, 283–286, 289; spectrophotometry, 302, 303; bile, 420; gases of bird egg, 636; urea determination, 690; oxygen tension, 859, 861, 862; air bladder, 867
Hürthle, K., 264
Hugounenq, L., biliverdin, 434; hæmatogen, 629; clupervin, 636; ash of milk, and of child, 666
Huiskamp, W., fibrinogen and fibrin formation, 253, 254, 256, 258, 320; nucleoprotein in blood, 258; thymus histone, 366, 367
Hulett, G. A., 29
Hultgren, E. O., utilization of nutr tives, 531, 535, 542, 543; diets, 932, 936
Hummelberger, F., 126
Humnicki, V., 448
Hundeshagen, F., 122, 242
Hunter, A., 106, 111, 134, 758
Hupfer, Fr., 721
Huppert, H., Schütze's rule, 57; digestion products, 133; glycogen, 307, 391; bile pigment reactions, 432; pepsin determination, 469; flesh, 601; urea, 686; uroleucinic acid, 739; urine albumin, 794; laiose, 814; acetone, 825
Hurtley, W. H., 287, 739
Hurwitz, S. H., 253
Husson, 671
Hutchinson, Rob., 553
Hybbinette, S., 748

Ibrahim, J., 497
Ide, 578
Ignatowski, A., 827
Inagaki, C., serum albumin, 262; serumglobulin, 270; blood corpuscles, 341; protein absorption, 528; muscle rigor, 590
Inoko, Y., 277
Inouye, K., 179, 574, 748, 749
Inoye, Z., 476
Irisawa, T., 334, 749
Ishihara, H., 423
Ito, M., 791
Iversen, A., 621, 623
Iwanoff, 763
Iwanoff, L., 182, 206, 508
Izer, G., autolysis, 43; uric acid, 704–706

Jaarswelde, G. J., 723
Jablonsky, J., 499
Jackson, H. C., 739
Jacobs, W. A., serine, 145; nucleic acid, 178–185; ribose, 211, glucothionic acid, 369; sphingosin, 611; cerebronic acid, 611
Jacobsen, A., 331
Jacobsen, C. A., 673
Jacobsen, O., 437, 438, 779
Jacoby, M., autolysis, 42, 45, 869; phosphorous poisoning, blood, 253, 255, 256; pepsin determination, 470; trypsin determination, 506; fertilization, 640; uric acid demolition, 706
Jacubowitsch, 458
Jaeckle, H., 233, 235, 559
Jäderholm, A., 285, 289
Jaeger, A., 867, 843
Jaffa, M. E., 936, 939
Jaffé, M., ornithine and ornithuric acid, 162, 783; bile pigments, 432, 433; urobilin, 521; creatine and creatinine, 574, 575, 693, 695; phenylsemicarbazide, 687; urethane, 692; uric acid, 703, 704, 708; indican in urine, 727–730; kyenuric acid, 739; urobilin, 740, 741, 744, 746; conjugated glucuronic acids, 751, 786; behavior of organic substances, 778, 779, 783, 786; furfurol, 784; thiophene, 784; guanidine acetic acid, 787
de Jager, L., 675, 677, 734, 756, 769
Jahnson-Blohm, G., 63
Jakowsky, M., 513
v. Jaksch, R., blood alkalinity, 309; urea, 333; brain, 605; volatile fatty acids, 748; melanin, 799; pentoseuria, 816; acetone, 818
Jalowetz, E., 226
Jamieson, G. S., 123
Janney, N., 768

Jansen, B. C. P., 491, 581
Jappelli, G., 453
Jaquet, A., 277
Jastrowitz, H., 469, 716, 757
Jastrowitz, M., 208, 815
Jelinek, J., 407, 583
Jensen, P., 581, 587
Jerome, W., Smith, 753
Jerusalem, E., nucleic acids, 184; lactic acid, 488; melanins, 841–843
Jesner, S., 617
Jess, A., 619
Jessen-Hansen, H., 165, 809
Joachim, Jul., 259, 353, 357, 359
Jochmann, G., 307
Jodlbauer, A., 50, 552
Johansson, F., 757
Johansson, J., E., serumalbumin, 262; tissue and gas metabolism, 597, 898, 927–931, 936; digestion work and specific dynamic action, 930
Johnson, St., 691, 694
Johnson, T. B., protein sulphur, 79; thiopolypeptides, 87; nucleic acid, 185; cytosine, 194
Jolin, S., 376
Jolles, A., pentoses, 209, 210, 816; bile pigments, 431; milk, 664; urine acidity, 677; urinometer, 679; uric acid, 711; urobilin, 743; albumin in urine, 787; nucleohistone, 795; fructose determination, 814
Joly, 670
Jolyet, 851
Jones, D. B., 84, 106, 107
Jones, W., autolysis, 44; nucleoproteids, 175; nucleic acids, 182, 183; thymine, 195; purin substances and their enzymes, 368, 371, 373, 701–703, 706
Jonescu, D., 65
de Jonge, D., 846, 847
Jornara, D., 847
Josephsohn, A., 739
Jünger, E., 427
Jürgensen, E., 20, 97
Jungfleisch, E., 27, 585
Junkersdorf, P., 413
Juschtschenko, A., 373
Just, I., 339
Justus, J., 71
Juvalta, N., 778

Kaas, K., 78, 634
Kahn, R., 402
Kalb, G., 918
Kalberlah, Fr., 585, 818
Kalmus, E., 289
Kanitz, A., 506
Kapfberger, G., 53
Kareff, N., 253
Kashiwabara, M., 710
Kast, A., intestinal putrefaction, 519; urinary sulphur, 752; chlorine excretion, 758; halogen substituted methane, 777; perspiration, 848
Kastle, J. H., 56, 59
Kato, Kan, 636
Katsuyama, K., 161, 334, 582, 896
Katz, A., 744
Katz, J., 598, 599
Katzenstein, A., 926, 927
Kauder, G., 261
Kaufmann, M., glycogen, 397; sugar of blood, 398; sugar formation, 412; fat formation, 562; urea, 572; sugar utilization, 592; lactose, 670; urea formation, 684; metabolism experiments, 890
Kauffmann, M. (Frankfurt), 247, 360, 713
Kaufmann, Martin, 894, 912
Kaup, J., 595
Kausch, W., 395
Kautzsch, K., polypeptides, 87; glutamic acid, 147; proline, 154; adrenalin 379; digestion, 484, 513
Kaznelson, H., 464
Keller, A., 757

Keller, Fr., 525
Keller, W., 783
Kellner, O., utilization of nutriments, 531; protein catabolism in work, 595; asparagin, nutrition value, 912; diets, 932
Kelly, A., 839
Kempe, M., 157, 158
Kennaway, E. L., 83, 109, 700, 715
Kendall, A. J., 520
Kermauner, F., 521
Kerner, G., 694
Kiermayer, 211
Kiesel, A., 247
Kikkoji, T., 640
Kiliani, H., 197
Kirchmann, J., 911
Kirk, R., 739
Kirschbaum, P., 613
Kistermann, C., 806
Kitagawa, F., 608, 611
Kittsteiner, C., 847
Kjeldahl, J., methods for nitrogen determination, 687, 688
Klages, A., 427
Klatte, F., 205
v. Klaveren, H. K. L., 288
Klecki, K., 521
Klein, W., 675
Kleine, F., 752, 767
Kleiner, I. S., 717
Klemensiewicz, R., 476, 477
Klemperer, G., urochrom, 740, 741; oxalic acid, 773; protein metabolism, 903, 915, 934
af Klercker, O., 691, 692, 694, 815
Klingemann, F., 670
Klingemann, W., 484
Klingenberg, K., 779

Klug, F., tryptophan, 155; pepsin, 466, 472; trypsin, 502, 503; phosphoric acid excretion, 762
v. Knaffl-Lenz, E.., 124, 390
Knapp, K., sugar test, 214; sugar determination, 808, 811
v. Knieriem, W., cellulose, 510; urea formation, 682; uric acid formation, 704
Knöpfelmacher, W., 233, 559
Knoop, F., histidin, 159, 160; methylimidazol, 201; demolition of fatty acids, 774, 781; synthesis of amino acids, 775, 786; demolition of aromatic substances, 779, 781
Knop, W., 690
Knorr, L., 297
Kobert, H. W., 276
Kobert, R., cyanmethæmoglobin, 285; iron in urine, 770; melanins, 841
Kobrak, E., 662
Koch, W., lecithins, 241–245; cephalin, 248; brain analyses, 605, 607, 609, 612–614; milk, 663
Kocher, Th., 375
Kochs, W., 722
Kochmann, M., 902
Köbner, 440, 441
Koefoed, E., 647
Köhler, A., 600, 892, 912
Köelichen, K., 35
Koelker, A. H., 455
König, J., 599, 657–659
Köppe, H., blood corpuscles, 7, 8, 273, 305, 326; hydrochloric acid of stomach, 477
v. Körösy, K., digestion, 484, 513; parenternally introduced protein, 525; digestion blood, 527, 530
Köster, H., 650
Koettgen, E., 616
Kohler, R., 708
Kohlrauch, A., 776
Kojo, K., 795
Kolisch, R., 698, 795
Kondo, K., indol and skatol, 159; lactic acid, 333, 585; cholesterin, 385; chondroitin sulphuric acid, 548, 549; urinary phosphorous, 757
Korsen, G., 598, 929
Korányi, A., 13, 311, 351
Korkunoff, A., 910
Korn, A., 469
Korndöfer, G., 572
Korowin, 500
Kossel, A., protein nitrogen, 77, 78; nitration of protamines, 83; arginase, 89, 161, 574, 681, 682; histones, 107–109; protamines, 109–111, 137; protein hydrolysis, 122, 141, 145, 154; histidin, 159, 160; hexone bases, 161, 164; arginine, 161, 162; agmatine, 162; ornithin, 163; lysin, 163; nucleoproteins, 174; nucleic acids, 179, 185; purin bases, 186, 187, 190–193, 367, 386, 495; pyrimidine bases, 194, 195; primary and secondary cell constituents, 240; hæmoglobin, 277; nucleohistone, 307, 366; blood plates, 308; pus, 364; protagon, 606, 607, 610; cerebrosides, 607, 610; ichthulin, 630
Kossler, A., 725
Kostin, S., 287
Kostytschew, S., 184, 185
Kotake, Y., 179, 393, 394, 711, 780
Kowalewski, K., 132, 185, 704, 767
Kowarski, A., 572
Kraft, F., 17
Kramm, W., 695
Kranenburg, W. R. H., 476
Kraske, B., 333
Krasnosselsky, T., 369
Kratter, Jul., 560
Kraus, Fr., 384, 410, 411, 583
Krause, R. A., 692
Krauss, E., 730
Krauze, L., 83
Krawkow, N. P., amyloid, 171, 172; chitin, 839, 840
Kreglinger, G., 341
Krehl, L., 795
Kreis, H., 233
Kresteff, S., 477
Kreuzhage, C., 595
Krieger, H., 261, 263, 763
Krimberg, R., 575–578
Kristeller, L., 692
Kröber, E., 209
Krönig, B., 71
Krogh, A., oxygen in blood, 859, 862, 864, 867; microtonometer, 861, 864; carbon dioxide tension, 864; metabolism experiments, 868, 881
Krogh, M., 690, 862, 864, 866
Kronecker, F., 723
Krshyschkowsky, K., 463
Krüger, A., 79, 120, 230
Krüger, Fr., modification of proteins, 97; hæmoglobin, 280; leucocytes, 305; blood, 335, 336; spleen, 371; liver, 388; sulphocyanides in saliva, 455; milk, 654
Krüger, M., purin bases, 186, 188; in feces, 520; in urine, 711–715; ammonia, 768
Krüger, Th. R., 136, 578, 598
Krug, B., 918
Krukenberg, F. C. W., keratinalbumoses, 113; skeletins, 121; cornein, 122; cornicrystallin, 123; hyalogens, 170; 171; lipochrome, 267; hæmoerythrin, 303, 304; muscle extractives, 572, 577; bird egg, 636; uroostealith, 834–5; bird feathers, 843
Krumbholz, C. J., 536
Krummacher, O., sugar formation, 412; work, 595; calorific power, 889; protein metabolism, 909; nutritive value of gelatin, 911

Krzemecki, A., 82
Kudo, T., 476, 479, 506
Kübel, F., 457
Kühling, O., 784, 785
Kühne, W., enzymes, 40; neurokeratin, 112, 605, 614; gelatin, 119; albumoses, and peptones, 128, 129, 132, 135–139; paraglobulin, 258; hæmatoidin, 301; glycogen, 390; gastric digestion, 472, 485; pancreas and its enzymes, 500, 502, 503, 508, 509; fat emulsion, 536; muscles, 566–568, 589; smooth muscles, 602; pigments of the eye, 615, 616; corpora lutea, 623
Külz, C., 355
Külz, E., cystine, 147; pentoses, 208, 815; isomaltose, 225; glycogen, 390–394, 397, 592; diabetes, 400, 403; saliva, 454, 456; gastric juice, 476, 477; pancreatic diastase, 500; gases of milk, 664; conjugated glucuronic acids, 777, 785; oxybutyric acid, 826
Külz, R., 283, 857
Kueny, L., 215
Küster, F. W., 28
Küster, W., blood pigments, 283, 289–298, 430; bile pigments, 425, 428–431, 433, 434, 429
Küttner, S., 652
Kuhn, 617
Kuliabko, A., 494, 691
Kullberg, S., 205
Kumagai, T., 737
Kumagawa, M., fat determination, 238; fat formation, 562; sugar determination, 809; protein metabolism, 903, 915, 934
Kunkel, A. J., arsenic, 72; carbon monoxide blood test, 287; iron preparations, 340; bile, 435, 443; iron in urine, 770
Kuprianow, J., 582
Kurajeff, D., coagulose, 58, 135; halogen protein, 83; protamine, 109; tryptophan, 155
Kurbatoff, D., 237
Kurpjuweit, O., 520
Kusmine, K., 351
Kusumoto, Ch., 448, 449
Kutscher, Fr., protein nitrogen, 77; gelatin, 83; proteolysis, 106, 107, 122, 146; histone, 107, 108; protamine, 109; digestion products, 132; histidine, 159, 160; arginine, 161, 162; agmatin, 162, lysine, 163, 164; hexone bases, 164; aporrhegmen, 166; cytosin, 194; thymin, 368; erepsin, 493; guanidine, 507; choline, 507; intestinal digestion, 513; absorption, 527, 528; bases of meat extract, 575, 576; methylguanidine, 698; urinary bases, 757, 826, 828
Kuwschinski, P., 499
Kyes, P., 241

Laache, S., 342
Ladenburg, A., 621
Laidlaw, P. P., 301, 380, 843
Laitinen, T., 309
Lambling, E., 680
Lampé, A., 912
Lampel, H., 78, 126
Lanceraux, 406
Landau, A., 361
Landauer, A., 518, 899, 920
Landergren, E., utilization of nutriments, 531, 535; metabolism, 894, 903, 914; diets, 932, 935
Landois, L., 275, 343
Landolt, H., 841
Landsteiner, K., 69
Landwehr, H., 749
Lane-Claypton, J. E., 43
Lang, G., 484, 793
Lang, J., 151, 349
Lang, S., 703, 704, 776
de Lange, C., 242, 664
Lange, F., 818, 819
Langer, L., 235
Langgaard, A., 660
Langhaus, A., 230
Langhaus, Th., 301
Langheld, K., 81, 426
Langley, J. N., 457, 459, 477
Langstein, L., carbohydrates in proteins, 84, 168, 262; digestion products, 132; skatosin, 159; fibrin and leucocytes, 253; blood globulin, 260; rest nitrogen in blood, 267; serumprotein, 270; deamidation, 410, 411, 775; lactic acid, 583; ovoglobulin 633; ovalbumin, 633, 634; ovomucoid, 634; casein, 662; alcaptonuria, 735, 736, 739; C:N quotient, 772, 884; lactose in urine, 815
Lankester, E. R., 303, 304
Lannois, E., 533, 846
Lapicque, L., 371, 387, 388, 936
Lappe, J., 492
Laptschinsky, M., 619
Laqueur, E., autolysis, 43; lipase, 476; casein and rennin coagulation, 648–651
Laqueur, J., 340
Larin, A. M., 469
Larsson, K. O., 759
Lassaigne, J. L., 717
Lassar, O., 309
Lassar-Cohn, 422–425, 427, 435
Latarjet, A., 464
Latschenberger, J., bile pigments, 441, 442; iron in bile and liver, 443; protein absorption, 525
Latschinoff, P., 422–426
Lattes, L., 822
Laulanié, F., 563, 597, 927
Lauritzen, M., 769
de Laval, G., 656
Laves, E., 660
Laves, M., 397, 403, 404
Laveson, H., 479
Lavoisier, M., 926

Lawes, 563
Lawrow, D., coagulose, 58, 135; histone, 107, 108; digestion products, 132; histidine, 160; blood pigments, 288; conjugated glucuronic acids, 785
Laxa, O., 649, 651
Lazarus-Barlow, W. S., 351
Lea, Sh., 65
Leathes, J. B., lymph, 13; autolysis, 44; liver fat, 384, 385; protein absorption, 527; ovarial fluid, 626; uric acid, 700
Leavenworth, Ch., 78, 84, 163
Lebedeff, A., 384, 560, 561, 669
v. Lebedew, A., 205
Leclerc, A., 848
Leconte, P., 463
Ledderhose, G., 218, 839
Ledoux, A., 324
Leers, O., 285
van Leersum, E. C., 209, 221
Lefévre, K. U., 222
Lefmann, G., 692
Legal, E., 158, 823
Lehmann, C., fat formation, 563; metabolism in hunger, 893; in work, 926, 927; asparagin, food value, 912
Lehmann, C. G., 458, 632
Lehmann, Fr., 849
Lehmann, K. B., 288, 560
Lehndorff, H., 360
Leichtenstern, O., 338, 339
Leick, 559
Lemaire, F., 749, 815
Lenk, E., 423, 589, 590
Leo, H., liver fat, 384, 561; diabetes, 404; acidity in gastric juice, 489; laiose, 814; nitrogen deficit, 881
Lepage, L., 498, 499
Lepiné, R., glucuronic acids, 221, 331; pentoses, 264; sugar in blood, 264, 329, 330; glycolysis, 332, 333, 346, 407, 408; glycogen, 393, phlorhizin-diabetes, 400; absorption, 533; urinary sulphur, 752, 753; urinary phosphorous, 757; urinary poisons, 758; urine maltose, 814
Leponois, E., 758
Lerch, 619
Lesem, W., 606, 607
Lesnik, M., 778, 779
Lesser, E. J., 909–911, 917
Lesser, K. A., 350
Letsche, E., blood serum, 264, 266; methæmoglobin, 283; bile acids, 419, 420, 423
Leube, W., 787, 848
Leuchs, H., serine, 145; arabinose, 201; glucosamine, 218; oxyprolin, 155
Levene, P. A., autolysis, 44, 620; polypeptides, 89; nucleoalbumin, 104; albumin hydrolysis, 106, 107, 119, 134, 141, 143, 144, 146; plasteins, 135; tendon-mucin, 168, 544; nucleic acids, 178–185, 643; guanine, 190; pyrimidine bases, 194, 195, 507, ribose; 211; glucothionic acids, 307, 369, 387, 547, 643; spleen, 369; liver, 383; phlorhizin diabetes, 400; glycolysis, 408, 333; trypsin, 502, 503; brain protein, 604; cerebroside, 609, 611; sphingosin, 611; cerebronic acid, 611; ichthulin, 630; urea, 682, 689; creatin and creatinine, 692; lactic acid, 583
Levi-Malvano, M., 235, 236
Levison, L., 470
Levites, S., 78, 449–450, 476, 514
Levy, A. G., 309
Levy, H., 784
Levy, Ludw., 571
Levy, M., 557
Lewandowsky, M., 525
Lewin, Karl, 721, 728
Lewin, L., blood pigments, 281, 285, 286, 288, 289; hydroquinone, 727; urobilin, 745
Lewinsky, J., 269, 270, 721
Lewis, D. H., 53
Lewis, Th., 602
Lewy, B., 621
v. d. Leyen, E., 728
Lichtwitz, L., 66, 749
Liddle, L. M., 107
Lieb, Ch., 702
Lieben, A., 301, 306, 823
Liebermann, C., 447, 636, 843
Liebermann, H., 754
Liebermann, L., protein reaction, 100; lecithalbumins, 105; nucleins, 175, 176; hen's egg, 628, 630, 632, 637, 638; kidneys, 673; Guaiac test, 796
Liebermeister, G., 258
v. Liebig, J., mineral substances, 72; fat formation, 563; work and metabolism, 594, 596; urea, 686; diets, 932
Lieblein, V., 361, 684
Liebrecht, A., 82
Liebreich, O., 606, 844
Liechti, P., 726
Liepmann, W., 642
van Lier, E. H. B., 545
van Lier, G. A., 8
Lifschütz, J., oleic acid, 237; cholesterin, 447, 449; isocholesterin, 446; wool fat, 846
Likhatscheff, A., 785
Lilienfeld, L., nucleo histone, 108, 307; fibrin-ferment and blood coagulation, 256, 314, 316; blood plates, 308; thymus, 366, 368
Lillie, R. S., colloids, 16, 17, 31; salt action, 73
v. Limbeck, R., 309, 310
Limpricht, H., 572
Lindberger, W., 506, 517
Lindemann, L., 208
v. Linder, M., 843
Linden, S. E., 23, 26
Lindhard, J., 814
Lindvall, V., 112

Ling, A. R., 226
Linn, K., 445, 446
Linnert, K., 612, 613
Linossier, G., 675
Linser, P., 844
Lintner, C. J., 229
Lipliawsky, A., 824, 825
Lipp, A., 152
Lippich, Fr., metallic albuminates, 96; leucine, 143; excrements, 521; urein, 691; uramino acids, 786
v. Lippmann, E. O., 153
Lipschütz, A., 902
Lister, J., 313
Ljubarsky, E., 239
Ljungdahl, M., 402
Lloyd-Jones, E., 308
Lochhead, A. C., 606
Lochhead, J., 640
Loche, F. S., 72, 592
Lockemann, G., 307
Locquin, R., 144
Loeb, A., 415, 767, 333
Loeb, J., muscles, 9; Overton's theory, 10; enzymes, 70; antagonistic salt action, 72, 73; artificial fertilization, 639, 640; metabolism, 928; fundulustrials, 70, 73, 74
Loeb, L., 256, 319
Loeb, W., 37, 212, 597
Loebisch, Wilh., 643, 668
Loebisch, W. F., 434, 544
Löhlein, W., 469, 505, 506
Löning, H., 611
Lönnberg, J., 549, 673
Lönnqvist, B., 462
Loeper, M., 265
Lorcher, G., 474
Loeschcke, K., 392
Lötsch, E., 484
Loevenhart, A. S., enzymes, 56, 59, 71, 501, 502, 563
Loew, O., protein, 78, 82, 97, 126; sugar synthesis, 212
Löwe, S., 10
Löwenthal, S., 43
Loewenthal, W., 511
Loewi, O., phlorhizin-diabetes, 400; sugar formation, 412; protein synthesis, 529; urea formation, 682; allantoin, 718; conjugated glucuronic acids, 750; phosphorous metabolism, 761
Loewit, M., 314
Loewy, A., diamines, 163; blood alkalinity, 310; high altitudes, 341, 929; liver nitrogen, 387; work and metabolism, 595; acid action, 676; amino-acids in urine, 757; cystinuria, 827; gases of blood, 851, 852, 859, 861, 865; alveolar air, 860, 863, 865; metabolism, 888, 925–929; maintenance value, 897, 898
Loewy, E., 839
Lohmann, A., lysine, 164; choline, 246, 247, 379, 507; methylguanidine, 698; urinary bases, 757
Lohnstein, Th., 679, 804, 813
Lohrisch, H., 510
Lombardi, M., 96
Lombroso, U., 532, 535, 536, 539, 540
London, E. S., enzymes, 53; nucleic acids, 472; gastric lipase, 476; digestion, 482–485, 513, 514; pancreatic juice, 498; Eck's fistula, 529, 702; small intestines, 541; creatinine, 694; nuclein metabolism, 702, 705; starvation blood, 896
Long, J. H., lecithin, 244, 245; casein, 648, 649; urine nitrogen, 680; urinary coefficient, 771; elimination of alkali earths, 769
Longcope, W. T., 43
López-Suárez, J., 476
Lorrain-Smith, J., 343
Losev, G., 29
Lossen, F., 83
Lossen, J., 325
Lottermoser, A., 19, 23
Luchsinger, B., 394, 847
Luciani, L., 339, 892
Luckhardt, A. B., 348
Ludwig, C., bile, 441; gastric digestion, 485; pancreatic juice, 495; absorption of proteins, 526, 527; of sugar, 534; gases of blood, 850, 851
Ludwig, E., 239, 627, 709, 710
Lücke, A., hyalin, 171, 840; pus, 365; benzoic acid reaction, 722
Lüdecke, T., 243
Lüdecke, K., 247
Lüthje, H., sugar formation, 408, 410, 412; nitrogen retention, 530; oxalic acid, 715
Lukjánow, S., 415, 896
Lukomnik, I., 136
Lummert, W., 384, 563
Lundsgaard, Chr., 75, 310
Lunin, N., 900
Lusk, Gr., phlorhizin-diabetes, 399, 400, 407, 409, 412, 413; lactose in intestines, 532; in urine, 815
Lussana, F., 398
Luther, E., 808
Luzzatto, A., 815
Lyman, I. F., 702
Lyon, E. P., 73
Lyttkens, H., 265, 329, 332

Maas, O., 126
Maase, C., 774, 779, 780, 822
Macadam, J., 595
Macallum, A. B., 271, 340, 586, 769
Maccallum, A., (jr.), 711
MacCallum, J. B., 490
McClenden, J. F., 630
McClendon, 73
MacCollum, E. V., 902, 903
McCrudden, F. H., 557

Macfadyen, A., 41, 512
v. Mach, W., 703
Mackay, J. C. H., 800
Mackie, W. C., 573, 574
MacLean, H., 243, 247
Maclean, H., 407, 583
Macleod, J., glycoscuria, 402; bone marrow, 553; phosphocarnic acid, 578, 593; carbamic acid, 683, 691
MacMunn, Ch., A., hæmatoporphyrin, 295, 797, 843; echinochrom, 304; cholohæmatin, 435; myohaematin, 571; urobilinoid, 743; tetronerythrin, 843
Madsen, Th., 50, 57, 449–450
Magnanini, G., 159
Magne, H., 670
Magnier, 770
Magnus-Alsleben, E., 772
Magnus, G., 850
Magnus, R., 52, 524
Magnus Levy, A., spleen, 372; thyroid gland, 374, 376; liver, 382, 388, 389; diabetes, 413; salivary glands, 452; pancreas, 495; analyses of muscles, 599, 600; of brain tissue, 614; kidneys, 673; hippuric acid, 721, 722; fatty acids in urine, 748; benzoicglucuronic acid, 751; Bence-Jones' protein, 792; acetone bodies, 818, 820, 821, 826; respiration, 869; metabolism, 889, 923–925, 929, 897, 898
Maignon, F., 581
Maillard, L. C., creatinine, 693; indoxyl sulphuric acid, 727, 730; urinary sulphur, 752; urinary phosphorous, 762; ammonia, 766
Maillard, M. L., 71
Majert, W., 621
Makris, C., 660
Malcolm, J., 761
Malengreau, F., 242, 366, 367
Malenück, W. D., 109, 110
Malfatti, H., urine, purin bases, 715, amino-acids, 756; ammonia, 768, 769; phosphorous elimination, 788; fructose determination, 814
Mall, F., 121
Mallèvre, A., 510
Maly, R., oxyproteic acids, 82, 83; peptones, 137; bile pigments, 429, 431–433, 743; saliva, 453; hydrochloric acid secretion, 477; putrefaction, 517; luteins, 631
Manasse, P., 385
Manché, M., 592
Manchot, W., 279, 286, 298
Mancini, St., 208, 267, 740, 741
Mandel, J. A., glutamic acid, 147; nucleic acids, 179, 643; adenine-hexose compound, 180; guanylic acid, 183; naphthoresorcin reaction, 223; glucothionic acids, 307, 369, 387, 643, 673; spleen, 369; liver, 383; mammary glands, 668; reno-sulphuric acid, 673; urinary phosphorous, 757
Mandel, R., 407
Mandelstamm, E., 415
Mangold, E., 390, 894
Manicardi, C., 578
Mann, S., 612, 613
Manning, T. D., 491
Mansfeld, G., 265, 360, 377
Maquenne, L., starch, 227, 229, 230; cellose, 231; protein assimilation, 525; sugar absorption, 534; inosite, 579; sarcosin, 776; digestion work, 929, 930
Marcet, 188, 523
Marchetti, G., 560
Marchlewski, L., leaf and blood pigments, 276, 277, 296, 297; hæmin, 292; cholohæmatin and bilipurpurin, 435
Marcus, E., 259
Marcuse, W., 592, 593
Mares, F., 700, 702
Marfori, P., 773
Margulies, 806
Marie, P. L., 307, 364
Marino-Zucco, 246
Mark, H., 385
Markewicz, M., 767
Marquardsen, E., 519
Marshall, J., 735
Martin, C. J., fibrin ferment, 57, 256; toxin-antitoxin combinations, 68; blood coagulation, 323
Martin, S. H., 510
Martz, 846
Marum, A., 820
Marx, A., 756, 818, 822
Marxer, A., 406
Maschke, O., 94, 695
Masius, J. B., 429, 521
Massen, V., 683
Masuyama, M., 628
Mathews, A., arbacin, 108; protamine, 109, 623; lysine, 163; nucleic acids, 184; fibrinogen, 252, 253
Mathieu, E., 851
Matthes, M., 519, 795
Mattili, H. A., 892
Mauthner, J., 149, 445, 814, 912
Mawas, I., 253
Maximowitsch, S., 261, 262
May, R., 208
Mayeda, M., 172, 173, 462
Mayer, Arthur, 770
Mayer, A., 105
Mayer, E. W., 445, 448
Mayer, J., 920
Mayer, L., 532, 540
Mayer, Mart., 253, 270
Mayer, P., isoserin, 145; cystin, 147–149; mannoses, 201, 203; glucuronic acids, 221, 222; lecithin, 244; conjugated glucuronic acids, 331, 749, 750, 817; phosphatides, 385; deamidation, 410;

inosite, 579; oxalic acid, 716; indican, 728; skatoxyl-glucuronic acid, 732
Mayo-Robson, A. W., 414
Mays, K., 502, 503, 616
Mazurkiewicz W., 499
Meara, F. S., 131
Medigreceanu, Fl., 182, 277, 481
Meek, W. I., 253
Méhu, C., 351, 745, 746
Meigs, E. B., 589, 590, 603
Meillère, G., inosite, 579, 580, 818; urinary chlorine compounds, 758
Meinert, C. A., 531, 932
Meinertz, J., 385
Meisenheimer, J., 41, 205, 207, 213
Meissl, E., 563
Meissl, Th., 642
Meissner, F., digestion products, 471, 472; urea formatiom, 684; allantoin, 716, 717; hippuric acid, 720
Meister, V., 684
Mellanby, E., 573, 574
Mellanby, J., serum proteins, 259, 322; peptone-blood, 325; creatine and creatinine, 692, 694
Mendel, Lafayette B., enzymes, 52, 53; lymph formation, 351; saliva, 454; trypsinogen, 496; protein absorption, 525; creatine, 693; uric acid, 702, 705; allantoin, 717; kynurenic acid, 740; artificial feeding, 904, 906
Mendes de Leon, M. A., 662
Menozzi, A., 449–450
Menschutkins, 35
Menzies, J. A., 285
de Merejkowski, C., 843
v. Mering, J., urochloralic acid, 221, 777; sugar in blood, 264; blood from portal vein, 336, 532; glycogen, 394; phlorizin diabetes, 399, 400; pancreatic diabetes, 404; amylolysis, 456, 500
Merunowicz, J., 293
Mesermitski, 628
Messinger, J., 825
Messner, E., 529
Mester, Br., 519, 732, 752
Mett, S., 469
Meyer-Betz, Fr., hæmatoporphyrin, 295; bilirubin, 429, 744; urobilinoids, 743; urobilinogen, 744, 746, 747
Meyer, C., 388
v. Meyer, E., 23
Meyer, E., 440
Meyer, Erich, 735, 739, 783
Meyer, G., 531
Meyer, G. M., amino-acids in blood, 266; glucose, 408, 333; urea, 682, 689; lactic acid, 583
Meyer, H., 221, 703, 704
Meyer, Hans, 267
de Meyer, J., 408
Meyer, K., 64
Meyer, Kurt, 32, 396
Meyer, Lothar, 850
Meyer, P., 425, 429
Meyer-Wedell, L., 385
Michaelis, H., 265
Michaelis, L., cataphoresis, 20; colloid envelopment, 23; adsorption, 49–51, 97, 102; reaction of blood, 75; coagulation of proteins by heat, 20, 97; albumoses, 134; sugar of blood, 264, 265, 328, 329; butyrinases, 265; protein absorption, 525; milk, 657
Michaud, L., 401, 818, 822, 917
Michel, A., 261, 262, 263
Micheli, F., 139
Micko, K., 521, 578
v. Middendorff, M., 336
Mieg, W., 631
Miescher, F., protamines, 109–111; nuclein, 175; nucleic acid, 179; pus, 364, 365; spermatozoa, 622, 623; salmon metabolism, 902
Miethe, A., 281, 285, 286, 289
Migay, Th., 481
Miller, I. R., 701–703, 706
Millon, M. E., 99, 653
Mills, W., 715
Milroy, J. A., 290
Milroy, T. H., 175, 761
Minami, D., 501
Minkowski, O., blood alkalinity, 309; ascitic fluid, 358; glycogen, 397; sugar of blood, 298; phlorhizin-diabetes, 399, 400; pancreatic diabetes, 403–406; duodenal diabetes, 405; bile pigment, 442, 443; pancreas and absorption, 532; fat absorption, 535, 539, 540; lactic acid, 582, 749; uric acid, 703–705; allantoin, 717; histozym, 723; blood in diabetes, 856
Mitchell, P. H., 702
Mitjukoff, K., 626
Mittelbach, F., 254, 739, 793
v. Mituch, A., 637
Miura, K., 264, 393, 492
Miyamota, S., 467
Moeckel, K., 265
Möllenberg, R., 338
Moeller, J., 521
Möller, S., 825
Mörner, C., Th., albumoid, 114, 546, 549, 617; gelatin, 118, 120, 546; ichthylepidin, 121; gorgonin and pennatulin, 122; cornicrystalline, 123; proteins of anthozoa, 123; tyrosin test, 154; membranin, 171, 550, 617; fructose, 217; vitreous humor, 545, 616; cartilage tissue, 546–550; cornea, 550; mucoid, 545, 550, 551; bones, 555; crystalline lense, 617, 618; sugar in egg-white, 632; ovomucoid, 634, 635; perca globulin, 636; homogentisic acid, 738, chlorine determination, 760; gallic and tannic acid; 785; calcium diphosphate in urine sediment, 831

Mörner, K. A. H., sulphur of proteins, 79; cystine and cystein, 106, 113, 114, 147–149; thiolactic acid, 79, 113, 151; albuminate, 126, 569; pyro-racemic acid, 150; proteins of serum, 259, 260, 262; hæmin, 292, 293; blister fluid, 361; hydrochloric acid determination, 489; chondroitin sulphuric acid, 547, 673; muscle pigments, 571; urinary nitrogen, 680, 685; urea determination, 688, 689; fatty acids in urine, 748; urinary nubecula, 757; acetanilid, 779; albumin in urine, 787; nucleoalbumin of urine, 794; melanins, 799, 841; bile acids in urine, 800
Mohr, Fr., 488, 759
Mohr, L., diabetes and sugar formation, 406, 412, 413; purines in urine, 713
Mohr, P., 112
Moitessier, J., 287, 758, 792, 793
Moleschott, J., 932
Molisch, H., 215
Moll, L., 103
Monari, A., 593, 594, 698
Monod, O., 654
Moor, Ovid., 690, 691
Moore, J., 213
Moore, B., theory of Overton, 10; adsorpates, 12, 29; colloids, 16; glycoseuria, 402; bile and fatty acids, 511, 536; intestinal contents, 519; fat synthesis, 535; fat emulsion, 536
Mooser, W., 726
Moraczewski, W., excrements, 521; heart muscle, 599; pseudonuclein, 651; indican of urine, 728, 729
Morat, J., 592
Morawitz, P., fibrin and leucocytes, 147; serum proteins, 270; blood coagulation, 314, 315, 318–321, 324, 325; detection of albumoses in urine, 792
Morax, V., 724
Moreau, A., 867
Moreau, J., 229, 230
Morel, A., fibrinogen, 252, 253; serum lipase, 265; blood coagulation, 234; glycolysis, 332; hæmatogen, 629; milk, 654
Morel, I., 677
Morel, L., 501
Moreschi, A., 449
Morgen, A., 667
Morgenroth, J., 64, 68
Mori, Y., 531, 932
Moriggia, A., 847
Morishima, K., 382
Moritz, 479
Moritz, F., 354, 400, 401, 675
Moriya, G., 582, 606
Morkowin, N., 109
Morochowetz, L., 546
Morris, G. H., 41, 226, 229
Morse, H. N., 3
Moruzzi, G., 247
Moscatelli, R., allantoin, 355, 359; lactic acid, 593, 748, 749
Moscati, G., starch assimilation, 532; glycogen, 581, 591; placenta, 640
Mosen, R., 308
Mosse, M., pseudochylous appearance, 358; sugar in blood, 398; hydrochloric acid in stomach, 476; ethereal sulphuric acids, 724
Mott, F. W., choline in blood, 246; in cerebrospinal fluid, 360; diseases of the nervous system, 614
Mottram, W. H., 384, 385
Mouton, H., 65
Mühle, P., 136
Mühsam, J., 335
Müller, A., 15, 23
Müller, Alb., 489
Müller, Eduard, 307
Müller, Erich, 510
Müller, Ernst, 419, 421, 423
Müller, Fr., 136
Müller, Franz, 377, 378, 888, 926, 929
Müller, Friedrich, autolysis of pneumonic infiltrations, 45, 364, 869; glucosamine from proteins, 83, 84, 168, 169, 170, 626; high altitudes, 341; starvation (indican) 516; fat absorption, 538, 539; ethereal sulphuric acids, 724; urobilin, 744; sulphur of urine, 752, 753; aniline, 779; acetone bodies, 818; feces nitrogen, 881
Müller, Joh., 580, 592, 628
Müller, Jul., 727
Müller, Max, 600, 721
Müller, Martin, 578
Müller, Paul, 448, 521
Müller, Paul, Th., 252, 253, 270, 553
Müller, W., 609, 610
Müntz, A., 669
Münzer, E., 682, 685
Müther, A., 210
Muirhead, A., 683
Mulder, G. J., 111
Munk, I., chyle and lymph, 346–349; sulphocyanides, 455, 456, 751; intestinal contents, 519; absorption of protein, 525, 526; of sugar, 534; of fat, 535, 537, 538; fat synthesis and fat formation, 560, 563; work and metabolism, 595; smooth muscles, 602; milk, 655, 656; urea, 682; phenol elimination, 725; phosphoric acid elimination, 762; bile pigment reaction, 801; starvation metabolism, 895, 896; nutritive value of gelatin, 911; of albumoses, 912; of asparagin, 912; protein requirement. 915; water and metabolism, 920
Murlin, J. R., 911
Murray, Fr. W., 500
Murschhauser, H., 923
Musculus, F., amylolysis, 229, 456, 500; urochloralic acid, 777; urease, 829

Myers, V., 195, 692, 693
Mygge, J., 793
Mylius, F., starch iodide, 228; bile acids, 419, 422–425

v. Nägeli, C., 227
Nägeli, K., 5
Nägeli, O., 676, 677
Nagano, J., 491, 532, 533
Nakaseko, R., 393
Nakayama, M., 182, 493, 801
van Name, W. G., 118, 121
Nasse, H., muscle experiments, 9; blood, 336, 337; lymph, 349; spleen, 371
Nasse, O., proteins, 78, 99, glutin, 120; dextrins, 230; glycolysis, 332; glycogen, 390, 391, 590–592; saliva, 456; 457; musculin, 568, 570; oxidations, 877
Naunyn, B., glycogen, 394; bile pigments and liver, 442, 443; demolition of aromatic substances, 778, 779
Nawratzki, 360
Nebelthau, E., 393, 799
Necker, F., 791
Nef, J. U., 213, 214
Neilson, C. H., 37, 53, 454
Neimann, W., glucuronic acids, 221, 222, 750, 751
Nelson, L., 109, 110
Nencki, L., 779
Nencki, M., protein sulphur, 78; tryptophan, 155; skatol acetic acid, 155; indol, 157; blood pigments, 276, 277, 280, 291-295; hæmatoporphyrin, 295; diabetes, 403; gastric juice, 465–467, 476, 477, 485; enzymes of the stomach, 475; cleavage of esters, 501; intestinal digestion, 511, 512; intestinal putrefaction, 514; reaction in intestine, 519; ammonia, 528, 683, 768; urea, 572, 682–684; carbamic acid, 683; urosein, 733, 740; urobilinoids, 743; demolition of aromatic substances, 778, 780, 785, melanins, 841
Neppi, B., 505
Nerking, J., lecithin, 241, 244; glycogen, 392; bone marrow, 553; milk, 663
Nernst, W., permeability of membranes, 9; division rule, 27; diffusion, 36; toxin-antotixin reaction, 69; gas chains, 74, 272
Nersessoff, N., 744
Neubauer, C., creatin, 575; creatinine, 691; ammonia, 766
Neubauer, E., 245, 404
Neubauer, O., protein reaction, 100; acids of alcaptonurics, 736, 738, 739; demolition of amino-acids, 775, 779, 780, 786; conjugation of glucuronic acid, 777; acetone formation, 780, 818, 822
Neuberg, C., autolysis, 43; putrefactive products of proteins, 82; gelatin, 83; artificial phosphoproteins, 105; isoleucine, 144; isoserine, 145; oxyaminosuccinic acid, 147; cystine, 147–149; proline, 154; tryptophan, 157, 158; diamine formation, 163; amyloid, 171, 172; nucleic acids, 178, 179, 182, 183; sugar demolition, 200; mannoses, 201, 203; pentoses, 203, 208–211, 815; sugar-free fermentation, 206; glucose, 215; galactose, 216; lævulose, 217, 218, 355; glucoseamine, 219, 626, 629; aminoaldehyde, 219, 220; glucuronic acids, 221, 223, 749–751, 817; amino-acids in blood, 267; glucothionic acid, 369, 673; melanin, 380; softening of liver, 386, 387; glycogen, 393; deamdiation, 410, 411; cholesterin, 445, 447; chondrosin, 548; inosite, 579; lactic acid, 583; lactose, 655; renosulphuric acid, 673; urine, 678; heteroxanthine, 713; phenol determination, 725, 726; skatoxyl-glucuronic acid, 732; amino-acids in urine, 756; mineral metabolism, 758, 769; tataric acid, 774; phenylhydrazine test, 806; acetone 818; cystinuria, 827; tyrosinase, 843; mineral metabolism, 899, 901, 902
Neumann, Alb., nucleic acids, 179, 184, 185; pyrimidine bases, 194, 195; orcin test, 209; iron in urine, 770; phenylhydrazine test, 806
Neumann, E., 443
Neumann, Jul., 338
Neumann, O., 921, 934
Neumann, R., 920
Neumann, Walt, 136
Neumeister, R., keratins, 112; albumoses and peptones, 128–131; tryptophan, 155; dextrins, 230; glycogen, 391; protein assimilation, 525; ovomucoid, 634
Neusser, E., 797
Nickles, J., 635
Nicloux, M., 56, 264
Nicolaier, A., 710
Niemann, A., 532, 539
Nierenstein, E., 469
Nilson, G., 228
Nilson, L. F., 658
Nishi, M., 404
Njegovan, Vl., 245
Le Nobel, C., 295, 743, 797
Noel-Paton, D., lymph, 349; liver, 384, 385; glycogen, sugar formation, 399; bile, 414, 438; creatine metabolism, 573-575; lactose, 670
Noguchi, H., 449–450
Nogueira, A., 673
Nolf, P., osmotic pressure, 13; fibrinogen 252, 253; fibrinolysis, 255, 256; albumoses in blood, 263; blood coagulation, 318, 319-322, 324, 325, 320; saliva, 452; absorption, 526, 527; carbamic acid, 683, 691
Noll, A., 184, 614, 535

v. Norden, C., spectrophotometry, 302; diabetes, 401, 404, 405, 412; liver and urinary nitrogen, 685; ethereal sulphuric acids, 724; albumin in urine, 787; acetone bodies, 818; metabolism, 915, 919
v. Noorden, K., 333
Nothwang, Fr., 899
Noskin, J., 375
Novi, J., 435, 459
Novy, F., 126
Nowak, J., 600, 868, 881
Nürenberg, A., 376
Nürenberg, A., 56
Nussbaum, M., 860, 864, 865
Nuttal, G., 516
Nylander, E., 214
Nylén, S., 457,

Obermayer, Fr., protein precipitation, 102; globulins, 103; bile pigments, 434, 801, 802; indican detection, 729, 730
Obermüller, K., 445, 446
Odake, S., 905, 906
Oddi, R., 511, 675
Odenius, R., 643
Oertel, H., 757
Oertmann, E., 41
Oerum, H. P., (sr.), 627, 911
Oerum, H. P. T. (jr.), 342, 437, 730
Oesterberg, E., 680, 693
Offer, Th. R., pentose-amine, 219; glycogen, 396; chitin, 839; alcohol, 921
Offringa, J., 282
Ofner, R., 217, 218
Ogata, M., 485
Ohta, K., 774
Oidtmann, H., 366, 368, 870
Oker-Blom, M., 8, 377
Okunew, W., 135
Olinger, J., 529
Oliver, G., 379
Ollendorff, G., 203
Olsavszky, V., 762
Omeliansky, V., 510
Omi, K., 492, 526
van Oondt, 401
Opie, E. L., 307
Oppenheimer, C., serumalbumin, 261; parenteral protein assimilation, 525; respiration, 868; oxydation enzymes, 875; nitrogen elimination, 881; surface rule, 923
Oppenheimer, Max, 583
Oppenheimer, S., 583
Oppler, B., 329, 513
Orbán, R., 492
Orgler, A., acetone, 83, 818; chondrosin, 548; uric acid, 638
Orndorff, W. R., 428, 430, 431
Orton, K., 738, 739
Osborne, Th. B., proteins, 77–79, 84, 104, 106–108, 163; polypeptides, 89; nucleic acids, 179, 185; ovovitellin, 628, 629; protein of white of egg, 633, 634; calorimetry, 885; phosphorous metabolism, 903; artificial nutrition, 904, 906
Osborne, W. A., 581
Ostertag, 659
Ostwald, Wilh., 28, 29; catalysis, 33, 35, 36
Ostwald, Wo., 15, 31, 279
Ostwald, A., halogen protein, 82; thyroid glands, 373, 375, 376; di-iodotyrosin, 508; urinary globulin, 791
Otori, J., mucin, 169; transudates, 355; guanidine, 507, 574; pseudomucin, 626
Ott, A., 769
Otte, P., 486
Otto, J. G., sugar of blood, 265; blood pigments, 277, 283, 284. 302; blood, 328, 329, 335, 338, 339, 532; skatoxylsulphuric acid, 732; sugar determination, 811
Overton, E., plasmolysis, 6, 8; muscle experiments, 9, 588; amphibians, 9; theory of permeability, 9–11
Owen, Rees, 347

Paal, C., proteins, 78; gelatin peptones, 120; alkali albuminate, 126; peptone, 130, 131, 137
Pacchioni, D., 381
Pachon, V., blood coagulation, 324; stomach extirpation, 485, 486; trypsinogen, 496
Paderi, 332
Padtberg, J. H., 838
Pagés, C., blood coagulation, 251, 316, 317; rennin action, 650; milk, 667
Pagniez, Ph., 314, 315
Paigkull, L., exudates, 353, 354, 357, 358, bile, 417
Painter, H. M., 130, 457
Panek, K., 753, 754, 763
Panella, A., 266, 578, 602
Panormoff, A., 581, 633, 635
Panum, P., serumcasein, 258; starvation blood, 338, 339, 896; transfusion, 340, 344; amount of blood, 343; nitrogen elimination, 910, nutritive value of gelatin, 911
Panzer, Th., proteins, 83; chylus, 347, cerebrospinal fluid, 360; bile, 423; colloid, 625, 626
Pappenhusen, Th., 484
Paraschtschuk, S., 669
Parastschuk, S. W., 474
Parcus, E., 610
Parke, J. L., 632, 635
Parker, W. H., 16, 536
Parmentier, E., 660
Parnas, J., 248, 583
Partridge, C. L., 371, 702
Paschutin, V., 350, 492

Pascucci, O., 273, 274
Pascheles, W., 776
Pasqualis, G., 749
Pasteur, L., 40, 41, 516
Patein, G., 808
Patten, A. J., 79, 149, 160
Patten, J. B., 457
Paul, Th., 71, 707
Pauli, W., colloids, 17, 18, 20, 25, 30;-proteins, 95, 97; gelatin, 31, 119
Pauly, H., histidin, 82, 159–161, adrenalin, 379
Pautz, W., 361, 492, 617
Pavy, F. W., carbohydrate groups in proteins, 84, 396; isomaltose, 264; glycogen, 392, 398; sugar in blood and diabetes, 400; self-digestion of stomach, 486; work and metabolism, 595; sugar determination, 808, 809
Pawlow, J. P., secretion of enzymes, 52, 53; Schütze's rule, 57; bile fistula, 414; saliva, 454; stomach and gastric juice, 461–463, 465, 468; stomach enzymes, 474; pyloric reflex, 481; intestinal juice, 490; pancreatic juice and enzymes of pancreas, 495–498, 499, 501; digestion in intestines, 513; ammonia in blood, 683; Eck's fistula, 684
Payer, A., 338
Pearce, R. G., 399
Peiper, G., 309
Pierce, G., 71
Péju, P., 253
Pekelharing, C. A., cataphoresis, 50, 51; fibrin ferment and blood coagulation, 256, 257, 316, 317, 319, 320; nucleoproteins, 258, 569; stomach enzymes, 466–468; creatin and creatinine, 594, 692, 693
Penny, E., 725
Penzoldt, F., 402, 824
Pernou, M., 371
Perrin, J., 15, 20
Peschek, E., 912
Petersen, P., 599
Petit, A., 758
Petrowa, M., 416
Petry, E., 651
v. Pettenkofer, M., bile acid test, 418; fat formation, 560, 561; work and metabolism, 594, 596, 597; respiration apparatus, 868, 869; metabolism experiments, 879, 881, 908, 926; diets, 932
Pittibone, C., 690
Petrone, A., 315
Petzsch, E., 902
Pfaff, F., 414
Pfannenstiel, J., 620
Pfaundler, M., 132, 133, 681
Pfeffer, W., 2, 3
Pfeiffer, E., 662, 665
Pfeiffer, L., 841
Pfeiffer, Th., 918
Pfeiffer, Wilh., 705
Pfleidener, 470, 471
Pflüger, E., oxydations, 41; life of cells, 45; carbonic acid of lymph, 347; glycogen, 390, 392, 393, 394, 413, 549; diabetes and sugar formation, 405, 408, 412, 413; duodenum and diabetes, 405; gases of saliva, 453, 857; bile and fatty acids, 511, 536; fat formation, 561, 562; muscle metabolism, 591, 595, 597; gases of milk, 657, 857; urinary nitrogen, 680; urea determination, 690; sugar tests, 803, 805; sugar determination, 811; gases of blood and respiration, 850, 851, 854, 858, 866–868; N:C quotient in urine, 884; protein metabolism, 904, 908, 909; external temperature and metabolism, 929; protein allowance, 934
Phisalix, C., 847
Picard, J., 616, 617
Piccard, 109
Piccolo, G., 301, 623
Pick, A., 470
Pick, E. P., albumoses and peptones, 131, 133–135, 139; serumglobulins, 259; peptozym, 325; thyreoidea and adrenalin-glycosuria, 376
Pick, F., 399
Pickardt, M., 355, 549, 550
Pickering, J. W., 85, 86, 323
Picton, H., 22, 26
Pierallini, G., 773
Piettre, M., stroma of blood corpuscles, 275; blood pigment, 281, 285; hæmin, 292; hyoglycocholic acid, 421; melanin, 842
Pigeand, J., 354
Pighini, G., 613
Piloty, O., blood and blood pigments, 220, 277, 290, 292–298, 295, 297; bile pigments, 429, conjugated glucuronic acids, 751
Pilz, O., 137
Pilzecker, A., 440
Pimenow, P., 462
Pincussohn, L., 23
Pineles, Fr., 376
Pinkus, S. N., proteins and their crystallization, 82, 95, 263, 633, 634
Piontkowski, L. F., 463
Piria, 153
Planer, J., 486
Plattner, E., 418
Plaut, M., 756
Playfair, 932
Pletnew, D., 493, 532
Plimmer, R. H., Aders, enzymes, 53; gelatin hydrolysis, 119; nucleoproteins, 174, 630; livetin, 629; ichthulin, 630; hatching of the egg, 637; casein digestion, 652; uric acid, 702
Plósz, P., blood corpuscles, 274; liver 382, 383; urinary pigments, 740;

proteid in urine, 787, albumoses, nutrition value, 912
Poda, H., 521
Poduschka, P., 717
Poehl, A., 517, 621
Pohl, J., dextrin, 230; globulin determination; 261, 793; liver, 383; acid poisoning, 676; urea, 682; allantoin, 716, 717; oxalic acid, 773; demolition of fatty acids, 774; phthalic acid, 778
Poleck, 631
Policard, A., 324
Polimanti, O., 561
Politis, G., 912
Pollak, H., 105
Pollak, L., 505, 776
Pollitzer, S., 912
Polwzowa, W., 482, 514
Ponfick, E., 343, 344
Ponamarew, 489, 490
Pons, Ch., 364, 548, 757
Popel, W., 339
Popielska, Helene, 463
Popielski, L., enzymes, 53, 324; saliva, 454; pancreatic juice, 496, 498
Popowsky, N., 158
Popper, H., glycogen, 395; bile pigments, 434, 801, 802; pancreatic juice, 500, 509
Porcher, Ch., lactose, 670; indican of urine, 728–732; skatol red and uroroscin, 733; uroerythrin, 748; phthalic acid, 778
Porges, O., 245, 259
Porteret, E., 393
Portier, P., 407, 532
Posner, C., 620, 621, 787
Posner, E. R., 169, 472, 606
Posselt, L., 122
Posternak, S., 578, 579
Pottevin, H., 59, 226
Pouchet, A. G., carnine, 577, 711; urinary poisons, 757; lungs, 870
Poulet, V., 869
Poulsen, W., 550
Poulsson, E., 572
Pozerski, E., 65, 498
Pozzi-Escot, E., 877
Prausnitz, W., 400, 521, 894
Prayon, I., 693
Pregyl, Fr., keratins, 112, 114; koilin, 115, 124; carbon monoxy hæmoglobin, 290; dehydrocholan, 418; bile acids, 423, 426; intestinal juice, 490, 491; colloid, 626; ovalbumin, 634; oxyproteic acid, 753; polypeptides in urine, 756; C:N quotient, 772
Presch, W., 752, 753
Preti, L., 64, 71, 706
Preusse, C., phenols in urine, 725–727; behavior of aromatic substances in animal body, 778, 779, 786
Prevost, J. L., 541, 684
Preyer, W., 288, 642
Preysz, K., 762
Pribram, E., 778
Pribram, H., 106
Pribram, R., 766
Pridgent, G., 242
Pringle, H., histopeptone ,109; protamines, 109, 110; absorption, 525, 528, 530; blood coagulation, 320
Pringsheim, H., 230, 510
Pristley, J. H., 37
Prochownik, L., 642, 643
Pröscher, F., 431, 659, 667
Profitlich, W., 389
Prutz, W., 728
Prym, O., 372, 496
Przibram, H., 571
Pugliese, A., 319, 476
Pulvermacher, G., 212
Pupkin, Z., 309
Pyman, F. L., 160

Quagliarello, G., 675
Quevenne, Th., 348, 349
Quincke, G., 23, 646
Quincke, H., 301, 340
Quinquand, Ch., urea, 333, 335; muscle work, 592; fatty acids in urine, 773
Quinton, R., 13

Raaschou, C. A., 183
Rabinowitsch, A. G., 484
Rachford, B. K., 501, 506, 511
Radenhausen, P., 646
Radziejewski, S., 559, 560
Radzikowski, C., 462
Raehlmann, E., 616, 617
Raineri, G., 642
Rakoczy, A., 475
Ramsden, W., 30, 97
Ranc, A., 267
Ranke, H., 700
Ranke, J., 344
Ransom, H., 449
Raper, H. S., 136
Rapp, R., 41
Raske, K., 140, 148
Rauchwerger, D., 445, 447
Raudnitz, R. W., 644, 649
Reach, F., 381, 466, 597
Reale, E., 716, 752
Reemlin, E. B., 513
Reese, H., 756
Regnault, H. V., 849, 868, 881
Reh, A., 104, 366, 652
Rehfuss, M., 804
Rehn, E., 253
Reich, M., 770
Reich, O., 771
Reichel, H., 650
Reich-Herzberge, F., 508
Reid, E. W., 16
v. Reinbold, B., 283, 507, 808, 283
Reinecke, 558
Reiset, J., 849, 868, 881

Reiss, E., 260
Reiss, W., 619
Reitzenstein, A., 711
Rekowski, L., 786
Rennie, J., 494
Renvall, G., 769
Rettger, I., 257, 322
Rettger, L. F., 496
Reuss, A., 355, 357
Rewald, B., 179, 180, 210, 211
Reye, W., 254
Reynolds, J. E., 823
de Rey-Pailhade, J., 877
Rhodin, N. J., 43
v. Rhorer, L., 677
Ribaut, H., 877
Richards, A. N., albumoids, 116, 117, 118; hexone bases, 164; saliva, 455, 456
Richaud, A., 599
Richet, Ch., gastric juice, 465; fat formation, 458; urea, 682; uric acid, 706; thalassin, 846; respiration, 869; surface rule, 923; spleen, 372
Richter, Max, 621
Richter, P., 873
Richter, P. F., osmotic pressure, 13; amino-acids in blood, 267; softening of the liver, 386, 387, 685
Rieder, H., 881
Riegel, 464
Riegel, M., 647
Riegler, E., 290
Riehl, M., 869
Riess, L., 748, 749, 780
Riesser, O., 162, 611
Rinaldi, U., 572
Ringer, A. I., 721
Ringer, A. J., 412
Ringer, L., 316
Ringer, S., 72
Ringer, W. E., 50, 51, 675, 677, 708
Ringstedt, O. T., 675
Ritter, A., sugar in blood, 398; phlorhizin-diabetes, 400; fat absorption, 535; urinary fermentation, 829
Ritter, F., 439, 440, 445, 449
Rittausen, H., proteins, 94; leucinimide, 143; milk, 655
Riva, A., 740, 748, 799
Rivalta, F., 354
Roaf, H. E., lipoid theory, 10; adsorpate 12, 29; osmotic pressure of protein, 17, 17; lecithin, 246; glycoseuria, 402
Roberts, F., 279
Roberts, W., 509, 793, 812, 813
Robertson, T. B., lipoid theory, 10; protein salts, 95; crystallized albumin, 262; globulins, 263, 270; casein, 648, 649
Roch, G., 790
Rockwood, C. W., 757
Rockwood, D., bile and fatty acids, 511, 536; intestinal contents, 519
Rockwood, E. W., 525
Rodhe, E., 100
Rodier, A., 338
Rödén, H., 63
Roeder, G., 194, 195
Röhmann, F., amylolysis and diastase, 225, 265, 346, 456; glycolysis, 333; blood, 335; glycogen 393, 398; intestinal juice, 491, 492; bile and putrefaction, 518; absorption, 532-534, 538; muscles, 565, 589; casein, 649; phosphorous metabolism, 761, 902; sebum, 844; wool fat, 846; coccygeal glands secretion, 846, 847; oxydase action, 875; artificial feeding, 904
Röhrig, A., 591, 849
Rõse, B., 650
Rõse, C., 558
Rõse, Heinrich, 429, 295
Rösing, E., 877
Roger, G. H., 381
Roger, H., 457, 510
Rogozinski, F., 136
Rohde, A., 703
Rokitansky, P., 748
Rona, P., cataphoresis, 20; enzymes, 56; adsorption, 97, 102; thymushistone; 109; gelatin, 119; albumoses, 134; sugar in blood, 264, 265; butyrinases 265; calcium in serum, 269; sugar in blood, 328, 329; glucose, 333, duodenal, secretion, 489, 490; protein absorption, 525, 529; milk, 657; alcaptonuria, 735, 736
Ronchese, A., 768, 769
Ronchi, J., 849
Roos, E., 376, 761, 806, 807
Roosen, O., 698
Rorive, F., 217
Rosa, E. B., 869
Rose, W. C., 692, 693
Rosemann, R., gastric juice, 465, 479; milk, 670; nitrogen elimination, 910; alcohol, 921
Rosenbach, O., 749, 800, 827
Rosenbaum, A., 408
Rosenberg, Br., 401
Rosenberg, S., duodenal diabetes, 405; bile, 415; pancreatic juice, 497; putrefaction, 518; pancreas and absorption, 532, 535, 539
Rosenberger, F., 579, 816
Rosenfeld, B., 484
Rosenfeld, F., 728, 748, 912
Rosenfeld, G., fat and fat formation, 384, 560–562, 669; uric acid, 700; phenylhydrazine test, 806; acetone bodies, 819
Rosenfeld, L., 136
Rosenfeld, M., 290, 293
Rosenfeld, R., 264
Rosenheim, O., tryptophane reaction, 157; cerebrospinal fluid, 360; pancreatic lipase, 502; muscle-work, 592; protagon, 606–609

Rosenheim, Th., 915
Rosenqvist, E., 412
Rosenstein, A., chyle and lymph, 347, 348, 349; absorption, 526, 534, 537
Rosenstein, W., 403
Rosenthal, J., 868
Rosenthaler, L., 59, 66
Rosin, H., fructose, 217, 814; indican, 730; skatol pigments, 733; Rosenbach's urine test, 827
Rossi, O., 360
Rost, E., 920
Rost, Fr., 265
Rostoski, O., 792, 793
Roth, O., 267
Roth, W., 351
Rothberger, C. J., 683
Rothera, C. H., 77, 148, 824
Rothmann, A., 573, 692
Rotmann, F., 355
Rotschy, A., 295
Roux, E., 227
Rovida, C. L., hyaline substance, 274, 364
Rovighi, A., 517, 724
Rowland, S., 41–43, 371, 571
Rowntree, L. G., 183, 642, 643
Rozenblat, H., 462
Rubbrecht, R., 270
Rubner, M., protein sulphur, 79; sugar test, 215, 806, 815; utilization of nutriments, 531, 534, 535, 538; fat formation, 562, 563; milk, 664; metabolism experiments, 879, 885, 890, 894, 922, 923, 928, 929; nitrogen of excrements, 881; heat of combustion, 885, 886, 891, 932; utilization quota, 890, 916; protein catabolism, 910, 914, 916, 918; specific dynamic action, 917, 930; surface rule, 923, 924
Rubow, W., 586
Rudinger, C., 375, 406
Rüdel, G., 707
Ruff, O., 200, 203
Ruge, E., 515
Rulot, H., 255, 256
Rumpf, Th., 412, 767
Runeberg, J. W., 357
Ruppel, W. G., 606, 660, 844
Russel, 653
Russo, M., 839
Russo, Ph., 744
Rutherford, A., 838
Rutherford, Th. A., 112
Ryan, L. A., 603
Rywosch, D., 273

Sabanejew, A., 137
Sabbatani, S., 316
Sacharjin, 328
Sacharow, N., 135
Sachs, Fritz, papain, 65; pentoses, 210; nuclease, 182, 493, 508; hydrochloric acid secretion, 477; acetone formers, 818
Sachs, H., 64
Sachsse, R., 215, 228
Sackur, O., 648, 649
Saalkoff, W., 118
Sagelmann, A., 482
Sahli, H., 303, 325, 809
Saiki, T., 603, 748, 749
Saillet, hæmatoporphyrin, 295, 797, 798; urobilin and urobilinogen, 740, 743, 744, 746, 747
Sainsbury, 316
de Saint-Martin, L., 286
Saint Pierre, C., 867
Saito, S., 334, 582
Salaskin, S., digestion products, 132; plastein, 135; leucinimid, 143; blood alkalinity, 309; ammonia, 334, 336, 528, 683; erepsin, 393; urea, 682, 684; liver and acid formation, 685; uric acid formation, 703, 704
Salkowski, E., autolysis, 42; denaturing of proteins, 97; pseudonuclein, 104, 651; albumoses, 131; in urine, 792; putrefaction products, 141, 514; skatol carbonic acid, 155, 732, 733; indol, 158; pentoses, 208, 209, 815; glucuronic acid, 221; cerebrospinal fluid, 360; synovin, 362; liver-proteins, 383, 384; glycogen, 393; cholesterin, 447; saliva, 459; trypsin, 503; putrefaction, 517; corpse wax, 560; flesh, 600; dermoid cyst, 627; dextrose in white of egg, 632; ovomucoid, 634; casein, 651, 652; urea, 682, 684, 690; creatinine, 696; uric acid, 705, 709, 710; purine bases, 714; oxalic acid, 716, 773; allantoin, 716, 717; hippuric acid, 720; phenaceturic acid, 723; ethereal-sulphuric acids, 724; indican, 729, 730; urobilin, 745; urine, fatty acids, 748, 829; carbohydrates, 749, 815; sulphur compounds, 752, 753; adializable urinary constituents, 757, 795; urinary sulphur acids, 764; alkalies, 766; demolition of various substances, 776, 777, 783; hæmatoporphyrin, 797; sugar tests, 805, 806; acetone determination, 825; water and metabolism, 920
Salkowski, H., 141, 514, 720
Salomon, Georg, purin bases, 187; glycogen, 207; lactic acid, 334; urinary purines, 711–713
Salomon, H., 410, 818
Salomon, W., 682
Salomone, G., 78
Salomonsen, K. E., 740, 741
Samec, M., 141
Sammet, O., 825
Sammis, J. L., 936
Samuely, F., proteolysis, 107; amino-acids in urine, 756; cystine demolition, 776; melanoids, 843
Sandgren J., 265, 329, 332

Sandmeyer, W., pancreatic diabetes, 405; absorption, 532, 539, 540
Sandström, J., 374
Saneyoshi, S., 774, 777, 817
Sasaki, K., 757
Sasaki, T., 146, 147, 781, 784
Sato, T., 369
Satta, G., 681
Sauer, K., 402
Sauerbeck, E., 494
Savaré, M., 640, 641, 757
Savory, H., 792
Sawitsch, W., 496, 499
Sawjalow, W., 58, 135, 475
Saxl, P., 43, 387, 569
Scaffidi, V., ferratin, 384; iron in liver, 387; purine bases, in muscles, 572, 594, 603; uric acid, 706
Schade, H., 707
Schäfer, E., 379
Schaeffer, 50, 51
Schaffer, F., 78
Schaffer, Ph., 692, 711, 768, 827, 828
Schalfejeff, M., 292, 293
Schardinger, F., 581, 877, 230
Scheele, M. H., 454
Scheermesser, W., 136
Scheibe, A., 659, 664
Schemiakine, A. J., 477, 478
Schenck, Fr., 83, 398
Schenck, M., 422, 423
Schepowalnikoff, N. P., 495
Scherer, Fr., 925
v. Scherer, J., lymph, 348; inosite, 579, 580; meta and para-albumin, 625
Scheuer, M., 464
Scheunert, A., gastric digestion, 479, 482; duodenal secretion, 490; pancreatic stones, 509; cellulose, 510; peroxydase, 874
Schewket, O., 817
Schierbeck, N. P., saliva, 457; gases of stomach, 486; trypsin action, 506; excrements, 520; skin breathing, 849
Schiff, A., 469
Schiff, H., protein, 78; biuret test, 101; cholesterin, 448; urea, 686; uric acid, 709
Schiff, M., spleen, 372; liver, 381, sugar of liver, 398; bile, 416, 541; charging theory, 477, 496
Schindler, S., 368
Schittenhelm, A., nucleic acid, 182, 514; blood coagulation, 318, 323; deamidation enzymes, 371; purine bases, 520; urea formation, 682; uric acid and its formation, 373, 699–703, 705, 706, 709; amino-acids in urine, 756, 757, 827; ammonia, 767, 768
Schläpfer, V., 912
Schlatter, K., 485, 486
Schlesinger, A., 457
Schlesinger, W., 747
Schliep, L., 825
Schlösing, Th., 768
Schloessing, C., 22
Schloessberger, J. E., 665, 670
Schlossmann, A., 656, 658, 659, 663, 923
Schmey, M., 387, 599
Schmid, Jul., 702, 715
Schmidt, Ad., 513, 523
Schmidt, Albr., 621
Schmidt, Alex., blood coagulation, 256, 257, 305, 314–317, 319, 321–323; fibrinoplastic substances, 258, 319, 320; blood corpuscles, of frogs, 375; leucocytes, 305, 306; cell protein, 306, 307, 367; saliva, 453, 454; gases of blood, 852
Schmidt, C., serum, 270; blood, 328; lymph, 348; transudates, 353; saliva, 458; mucus of the mouth, 453, 454; gastric juice, 465; pancreatic juice, 499, 500; bile, 518; fat absorption, 538; osteomolacia, 555
Schmidt, C. H. L., 82
Schmidt, C. W., 870
Schmidt, E., 572
Schmidt, Ernst., 695
Schmidt, Fr., 818
Schmidt, Hub., 101
Schmidt, P., 711
Schmidt-Mühlheim, 251, 526, 910
Schmidt-Nielsen, Signe, 50
Schmidt-Nielsen, Sigvae, rennin, 50, 651; casein, 649, 650
Schmiedeberg, O., albumin crystals, 94; salmine, 110, 110; alkali albuminate, 126; onuphin, 171; nucleic acids, 179, 184, 185; nucleosin, 195; glucuronic acids, 221, 725, 730, 731, 785; ferratin, 383; chondroitin sulphuric acid, 547–549; urea, 682; hippuric acid, 722, 723; histozym, 723; melanin substances, 840, 841
Schmitz, E., 775, 780, 786, 818, 826
Schmitz, H., 136
Schmitz, K., 517, 519, 724
Schmutzer, I., 708
Schneider, A., 328
Schneider, E., 455, 739
Schöffer, A., 142
Schönbein, C. F., 766, 871
Schöndorff, B., urea, 333, 572, 664, 679 690; thyroidea, 376; glycogen, 390, 394, 396, 581; phlorhizin action, 400; uric acid, 701; sugar in urine, 803, 808; protein metabolism, 908, 909
Schöne, A., 208
Scholz, H., 728, 729
Schottelius, M., 516
Schotten, C., fellic acid, 427; intestinal putrefaction, 720; fatty acids in urine, 748, 773; damaluric and damolic acid, 758; behavior of aromatic substances in animal body, 780
Schoubenko, G., 79
Schoumow-Simanowski, E. O., 467, 477, 485

Schreiber, E., 701
Schreiner, Ph., 621
Schrener, M., flesh nitrogen, 600; calorific value of nitrogen, 892; protein feeding, 918
Schrodt, M., 553
v. Schröder, W., urea, 12, 333, 679, 682, 684; uric acid, 334, 703, 704
Schröter, F., 182
v. Schrötter, H., 130, 131, 137, 861, 865
Schryver, S. B., 43, 427
Schüle, 464
Schüle, A., 456
Schütz, E., Schütz's rule, 57; digestion products, 133; pepsin determination, 469; stomach movement, 479; lactic acid in urine, 749
Schütz, J., pepsin determination, 469; action, 471; hydrochloric acid, 489; bile and fat splitting, 502, 511
Schütze, A., 64
Schützenberger, P., 85, 86, 130
Schultze, B., 563
Schultze, E., 680
Schultze, F. E., 72
Schultzen, O., diabetes, 403; urea, 682, 683; lactic acid in urine, 748, 749; sarcosin, 776; behavior of aromatic substances in the animal body, 778–780
Schulz, Art., 299
Schulz, Fr. N., proteins, 79, 95, 98; oxyproteins, 83; histone, 108; galactosamine, 167, 173, 219; serumalbumin, 261, globin, 288; starvation blood, 339; premortal protein metabolism, 894
Schulz, H., 376, 546, 770
Schulz, C., 457
Schulz, E., products of hydrolysis of proteins, 141, 153; phenylalanine, 152; histidine, 160; arginine, 161; lysine 163; hexone bases, 164; vernine, 178–180; hemicelluloses, 231; phosphatides, 245; isocholesterin, 448
Schulze, E., 650
Schulze, F., 22
Schulze, H., 14, 21
Schumburg, W., 474, 889, 926
Schumm, O., blood, 342, 796; chyle fat, 347; sugar formation, 412; pancreatic cyst, 500
Schunck, C. A., 276, 277, 296, 631
Schunck, E., 740
Schur, H., uric acid, 700, 702, 706; urinary purines, 702, 713
Schurig, 387
Schuster, A., 531, 938
Schuurmanns-Stekhoven, 282
Schwalbe, E., 315, 384, 561
Schwann, Th., 414, 528
Schwarz, Carl, choline, 247; iodothyrin, 376; glycoseuria, 405; digestion, 483; secretin, 498
Schwarz, H., 216
Schwarz, Hugo, 116, 117, 118
Schwarz, Karl, 572, 575
Schwarz, L., 126, 477, 820
Schwarz, O., 402, 487, 825
Schwarzschild, M., 502, 508
Schweissénger, O., 790
Schwiening, 42, 43
Schwinge, W., 338
Scofield, H., 433
Scott, F. H., 174, 630, 637
Scott, L., 122
Scselkow, 851
Sebauer, R., 555
Sebelien, J., peptones, 130; milk, 644, 651, 652, 655, 656, 658; casein digestion, 651; carbohydrates in milk, 655
Seegen, J., sugar in blood, 264, 398, 592 597; sugar formation, 405; amylolysis, 456; respiration, 868; nitrogen deficit, 881; water and metabolism, 920
Seelig, P., 517
Seeman, J., protein substances, oxydation, 83; galactoseamine, 167; erepsin, 493; intestinal contents, 513; absorption, 527, 528; creatine and creatinine, 573; ovomucoid, 635
Segale, M., 72
Seisser, Ph., 702, 705, 709
Seitz, W., 381, 389
Seliwanoff, Th., 217
Selmi, 47
Semmer, G., 275
Senator, H., 729
Senter, G., 56
Sera, Y., 393, 394
Sertoli, E., 855
Sestini, F. and L., 699
Setschenow, J., 851, 853, 855
Shackell, L. F., 73
Shepard, C. W., 720
Shibata, N., 384, 561
Shimamura, T., 905, 906
Shinidzu, Y., 238
Shaw-Mackenzie, J. A., 502
Siau, R. L., 264, 400
Sieber, N., protein sulphur, 79; blood pigments, 280, 292, 293; hæmatoporphyrin, 295; glycolysis, 333; diabetes, 403; gastric juice, 361, 362, 485; stomach enzymes, 475; intestinal digestion, 512; Umikoff's reaction, 664; urosein,7 33, 740; urobilinoids, 743; nitrobenzaldehyde, 784; melanins, 841; lungs, 869
Siedentopf, H., 19
Siegert, F., 559
Siegfried, M.; peptone substances and kyrines, 121, 132, 133, 136–138, 146: reticulin, 121, 544; lysine, 163; carbamino reaction, 166, 855; jecorin, 385; phosphocarnic acid, 578, 585, 593, 598; orylic acid, 653; milk-nucleon 653, 663; phenol excretion, 725, 726
v. Siewert, A., 292

Signorelli, E., 757
Sikes, A. W., 663
Silbermann, M., 145, 147
Simaček, E., 407, 583
Simon, Fr., 606
Simon, G., 656, 658
Simon, L., G., 510
Simon, O., 45, 364, 870
de Sinety, L., 815
Sittig, O., 355
Siven, V. O., uric acid, 700; urinary purines, 713; protein metabolism, 903; 916, 934
Sivré, A., 482
Siwertzow, D., 241, 244
Sjöqvist, J., enzymes, 57; peptones 137; hydrochloric acid determination, 488, 489; intestinal concrements, 524; urinary nitrogen, 680, 685; urea determination, 688, 689
Skita, A., 145
v. Skramlik, E., 675
Skraup, Zd., protein nitrogen, 77, 78; hydrolysis of proteins, 119, 124, 146, 147; alkali albuminate, 126; oxyamino-acids, 165; carbohydrate, 215
Skworzow, W., 575, 576
Slansky, P., 585
Slavw, 379, 380
Slosse, A., 333, 684, 910
Slowtzoff, B., pentosan, 208; liver, 389; semen, 620; milk coagulation, 651; metabolism, 923, 926
v. Slyke, D. D., deamidation, 77, 78, 88; hydrolysis of albumin, 106, 107, 134, 141, 143, 144; plastein, 135; amino-acids in blood, 266; casein, 648, 649
van Slyke, L. L., 648, 649
Small, Fr., 707
Smétanka, F., 700, 702
Smirnow, A., 784
Smith, F., 847, 848
Smith, Herbert, 457, 551
Smith, Lorrain, 862, 863
Smith, W. J., 752
Socin, C. A., 395
Socoloff, N., 437
Söldner, F., milk, 647–650, 655–657, 662, 664–668
Sörensen, S. P. L., isoelectric point, 20; determination of the reaction, 74, 75; coagulation of proteins by heat, 20, 97; glycocoll, 140; phenylalanine, 152; proline, 154; arginine, 161; ornithin, 162, 163; formol titration, 165; hippuric acid, 723; urinary nitrogen, 756; ammonia, 768
Soetber, F., 763
Solera, L., 455, 456
Solley, Fr., 118, 120
Sollmann, T., chyle, 347; bile, 440; muscles, 566, 567, 570; uterine fibroma, 627
Sommer, A., 384
Sommerfeld, 438
Sommerfeld, P., 465
Sondén, K., respiration apparatus, 869; metabolism, 924, 925, 936
Sorby, H. C., 636
Soret, J., 281
Sourder, C., 502
Le Sourd, L., 314
Soudat, 665
Southgate, 506
Soxhlet, glucose, 216; galactose, 216; maltose, 225; fat formation, 563; milk, 647, 650, 656, 667, 669; sugar titration, 808, 809
Spack, Wl., 124
Spanpani, G., 669
Spangaro, S., 250
Spanjer-Herford, R., 455
Speck, C., 869, 926, 928
Spiegler, A., 899
Spiegler, E., 790, 841, 842
Spiro, K., colloids, precipitation, 25, 102; swelling, 31; diffusion, 32; gelatin, 119; glycocoll, 140; pyrasinedicarbonic acid, 220; serumglobulins, 259; blood coagulation, 318, 319, 324; rennin action, 650; urine acidity, 675; oxybutyric acid formation, 822
Spiro, P., 593
Spitzer, W., glycolysis, 333, 407; liver, 383; uric acid formation, 702, 875
Spriggs, E. J., 469
Spring, 14
Staal, J. Ph., 676, 733
Stade, W., 57, 476
Stadelmann, E., tryptophan, 155; icterus, 301; adrenal bodies, 377; bile, 414–416, 438, 441–444, 541; intestinal putrefaction, 519; nitrogen excretion, 685; ammonia, 768; pentosuria, 791; diabetes, blood, 856
Stadthagen, M., diamines, 47; adenine, 191; xanthocreatinine, 698; urinary sulphur, 752, cystinuria, 827
Städler, G., 434
Staehlin, R., 354
Stanek, V., 247
Stangassinger, R., 573, 692, 698
Stange, M., 233
Starke, J., 102
Starke, K., 262, 263
Starkenstein, E., 398, 402, 579, 817, 818
Starling, E. H., colloids, 16; enzymes, 57; lymph formation, 351; hormones, 375; enterokinase, 492, 496; secretin, 492, 498; intestinal enzymes, 492, 493; pancreatic erepsin, 493, 503; trypsinogen and trysin, 496, 497
Stassano, H., 496, 497
Stassow, B. D., 541
Stauber, A., 493
Stavenhagen, A., 41
Steel, M., 768
Steenbock, H., 723
Steensma, F. A., 158, 488, 732

Steiff, R., 724
Steiger, E., 161
Steil, H., 599
Stein, G., 445
Steinitz, Fr., phosphorous metabolism, 761; C:N quotient, 772, 884; lactose in urine, 815
Stenger, E., blood pigments, 281, 285, 286, 289; urobilin, 745
Stepanek, J. O., 628
Stepp, W., 905, 906
Stern, E., 35
Stern, Fr., 750
Stern, H., 441, 442
Stern, Heinrich, 621
Stern, L., uricolysis, 706; peroxidase, 873, 874; oxydation processes, 874, 875
Stern, M., 248, 630
Stern, R., 440, 724
Steudel, H., arginine, 162; hexone bases, 164; mucin, 169; nucleic acids, 179, 181, 183, 184; pyrimidine bases, 194, 195; glucoseamine, 219
Stewart, C. W., 471
Stewart, G. N., 326, 566, 567, 570
Steyrer, A., 571
Sticker, G., 455, 458
Stiles, P. G., 400, 457
Stock, J., 297, 297
Stockmann, R., 595
Stöltzner, H., 554
Stoffregen, A., 791
Stohmann, F., 510, 885
Stokes, 282, 289
Stoklasa, J., lecithin, 241; glycolysis, 407, 408, 509; lactic acid formation, 583; fermentation enzyme of milk, 653
Stokvis, B. J., bile pigments. 432, 433, 743; benzoic acid, 723; urobilin, 745, 792; hæmatoporphyrin, 797
Stolnikow, J., 793
Stolte, K., 220, 396, 682
Stoltzner, W., 554
Stolz, Fr., 379
Stomberg, H., 256, 322
Stone, W., 208
Stookey, L. B., 136, 394
Stoop, F., 145, 148
Storch, V., 646, 671
Strada, Fr., 364
Stradomsky, N., 773
Strashesko, N. D., 462
Strassburg, G., gases of lymph, 347; tension of the gases of blood, 861, 864, 868
Strassburger, J., 523
Strassuer, W., 877
Straub, W., 403, 899, 920
Strauch, F. W., 124
Strauss, Edw., 122
Strauss, H., fructose, 217, 218, 264; blood, 264; transudates, 264, 355, 358; bile, 437; lactic acid fermentation, 485; amino acid storage, 721, 722
Strecker, A., 242, 245, 422
Strickler, E., 658
Strigel, A., 870
Strohmer, F., 563
Strusiewicz, B., 912
Struve, H., 797
Strzyzowski, C., 293
Stübel, H., 894
Stütz, 791
Subbotin, V., 339, 921
Suckrow, Fr., 400
Sugg, E., 653
Suida, W., 445
Suleima, Th., 513
O'Sullivan, C., 54, 55, 57
Sundberg, C., 467, 468
Sunde, E., 655
Sundvik, E., purin bases, 188, 190; glucoseamine, 218; uric acid, 699; conjugated glucuronic, acids, 751, 777; chitin, 839; psylla alcohol, 846
Suter, F., 79, 113, 151
Suto, K., 238, 809
Suwa, A., 572, 780
Suzuki, W., cystine, 148; muscles, 572; crab meat, 578; phytase, 579; oryzanine, 905, 906
Svedberg, The., 14, 20
Svenson, N., 919
Swain, R. E., 159
Symmers, D., 757
Syniewski, V., 227, 229
v. Szontagh, F., 653, 658, 660, 662
Szydlowski, Z., 474
Szymonowicz, L., 379

Tachan, H., 145
Takahashi, D., 269, 329
Takaishi, M., 579
Takamine, J., 379
Takada, K., 787
Tallqvist, T. W., 914
Tammann, G., 60, 66
Tanaka, T., 371
Tangl, Fr., blood serum, 264, 271; blood analysis, 326; sugar in blood, 398; fat, 482; egg, development, 637, 638; casein, 648, 658; milk, 668; C:N quotient, 772, 884
Tanret, C., 200
v. Tappeimer, H., enzymes, 50; cellulose, 510, 515; bile acids, 541
v. Tarchanoff, J., 441, 632
Tarugi, B., 848
Tarulli, L., 675
Tawara, 932
Taylor, A. E., enzymes, 56; liver fat, 384; fat formation, 561; mineral starvation, 900
Tebb, Chr., reticulin, 121; glycogen, 392; amylolysis, 456; saccharate, 492; maltose, 500; protogon, 606–609; cholesterin in brain, 613
Tedesko, Fr., 676

Teeple, J., 428, 430, 431
Teichmann, L., 292
Tengström, B. St., 418
Terrat, P., 758
v. Terray, P., bile and putrefaction, 518, 520; oxalic acid, 716; lactic acid in urine, 748, 749
Terroine, E. F., 105, 500–503
Terry, O. P.. 454
Terunchi, Y., 682
Tezner, E., 455, 456
Thannhauser, S. J., 429
Theissier, 247
Thesen, J., 572, 729
Thévenot, 247
Thiele, O., 754
Thiemich, M., 384
Thierfelder, H., barium, 72; galactose, 216, 610, 611; glucuronic acid, 223; cephalin, 248; digestion and micro-organism, 516; protagon, 607, 608; cerebron and cerebroside, 608–611; yolk phosphatides, 630; mammary glands, 643, 669; sphingosin, 611; cerebronic acid, 611
Thies, Fr., 379, 380
Thiroloix, J., 406
Thiry, L., 490, 491
Thörner, W., 644
Thomas, K., 595, 611
Thomas, Karl, 903
Thomas, P., 492
Thomas, W., 488, 909, 911, 914, 917
Thompson, W. H., 574, 682
Thoms, H., 687
Thormählen, J., 799
Thudichum, L. W., phosphatides, 239, 240, 242, 245, 248; bilirubin, 431; brain phosphatides, 605, 607–609; cerebrosides, 609–611; sphingosin, 611; lutein, 631; paraxanthine, 714; urinary pigments, 740; alcohol in animal organism, 921
Thunberg, T., 873, 874, 876
Tichomirow, N. P., 468
Tidemann, F., 458
Tiemann, H., 652, 658
Tigerstedt, K., 762
Tigerstedt, R., respiration apparatus, 869; metabolism, 893, 894, 924, 925, 936; digestion work, 930
Tissot, J., 592
Tobler, L., 341, 484, 763
Toepfer, G., 530, 759
Tollens, B., carbohydrates, 197, 208–211, 216, 217; glucuronic acids, 223, 817; urea, 687; naphthoresorcin-reaction, 223, 817
Tollens, C., 724, 725, 750, 817
Tolmatscheff, 656, 662, 665
Tomasinelli, G., 848
Tomaszewski, Zd., 716
Tompson, E., 54, 55, 57
Torup, S., carbon monoxyhæmoglobin, 287, 288; globulins and carbonic acids, 853, 855
Totani, G., 161, 787
Tower, R. W., 121
Towles, C., 692, 693
Toyonaga, M., 388
Traube, J., 10, 11; absorption, 542; oxydation, 871
Traube, M., 1, 9
Traxl, W., 78
Treupel, G., 749, 808
Treves, Z., 78
Trier, G., 178–180, 240, 243
Trifanowski, D., 437
Trillat, A., 873
Tritschler, F., 773
Troisier, J., 744
Troller, J., 464
Trommer, C., 214
Trommsdorff, R., 877, 910
Trümpy, D., 847
Trunkel, H., 119, 120
Truthe, W., 230
Tschenloff, R., 910
Tschernoruzki, M., 307
Tscherwinsky, N., 563
Tschirjew, S., 344
Tsuboi, J., 339, 881
Tsuschija, J., 744, 747, 793
Tuczek, F., 459
Tüllner, H., 698
Türk, W., 146
Türkel, R., 585
Turban, K., 912
Turby, H., 491

Udranszky, L., diamines, 47; bile acids, 419, 800; urinary pigments and humus substances, 740; carbohydrates in urine, 749, 808; cystine, 827
Uffelmann, J., 488
Uhlik, M., 280, 282, 283
Ulrich, Chr., 226
Ultzmann, R., 832
Umber, F., albumoses, 133; nucleins, 175; transudates, 354; gastric juice, 464, 465; proteins of pancreas, 494; lævuloseuria, 814
Umikoff, N., 664
Underhill, F. P., protozym, 324; glycoseuria, 400, 402; saliva, 454; allantoin, 717; lactic acid in urine, 748, 749
Unna, P. G., 837, 844, 845
Ulpiani, C., 244, 699
Urano, F., 573, 587
Urbain, V., 851
Ure, A., 783
Usher, Fr., 37
Ussow, 506
Ustjanzew, W., 542

Vahlen, E., 425
Valenciennes, A., 603, 636
Valenti, A., 663

de Vamossy, Z., 379
Vandegrift, G. W., 545
Vandevelde, A. J. J., 653
Vanlair, C., 429, 521
Vasilin, H., 720, 723
Vassale, G., 374
Vaubel, W., 82, 99
Vauquelin, L. N., 717
Vay, Fr., 383, 592
v. d. Velde, A., 723
v. d. Velden, R., 724
Velichi, J., 602
Vella, L., 490
Veraguth, O., 910
Verhaegen, A., 465
Verneuil, 461
Vernois, M., 665
Vernon, H. M., erepsin, 57, 492, 493; white of egg, 65; pancreatic enzymes, 497, 500, 501, 503, 507, 509; muscle rigor, 590
Verploegh, H., creatin and creatinine, 573, 691–694, 698
Verzar, Fr., 404
Viault, P., 340
Victorow, C., 804
Vierordt, K., 302, 863
Vigno, L., 526
Vignon, L., 122
Vila, A., blood corpuscles, 275, 281; blood pigments, 285, 292; musculamine, 578
Ville, J., oxymethyl furfurol, 215; blood pigments, 281, 285; bile acids, 419; fat absorbtion, 539, 540; urinary chlorine compounds, 758; Bence-Jones protein, 792
Villiers, A., 758
Vincent, Sw., 376–602
Vines, S. H., 493, 502
Vinci, S., 314, 315
Virchow, R., 171, 301
Vitali, A., 796
Vitali, D., 758
Vitek, 407
Voegtlin, C., 507, 692, 694, 703
Völtz, W., 646, 912
Vogel, H., 372
Vogel, J., pentoses, 208, 815; isomaltose, 225; lactase, 492; amylolysis, 456, 500
Vogelius, 391
Voges, O., 680
Vohl, H., 579
Voit, C., glycogen, 390, 393, 395, 534; bile and putrefaction, 518; excrements, 520; absorption, 525, 537, 538; fat formation, 560, 561, 663; work and metabolism, 594, 597, 925; nitrogen in meat, 600, 883; urea formation, 684; phosphoric acid excretion, 762; standard numbers, 772, 915; lactose detection, 815; metabolism experiments, 879, 881, 920, 926, 929; starvation metabolism, 893, 903; water content of body, 898; mineral metabolism, 899; protein catabolism, 903, 906, 908–910, 913, 914; nutritive value of gelatin diets, 932–936, 938
Voit, E., glycogen, 395; bones, 554, 555; fat formation, 562, 563; calorific value of oxygen, 889; of nutritive substances, 892; starvation metabolism, 894, 896 897; nitrogen excretion, 910; vegetable diet, 915; protein minimum, 917; surface rule, 923
Voit, Fr., galactose fermentation, 216; thyroidea and metabolism, 376; glycogen formation, 395; sugar elimination, 395, 534; feces formation, 521; lactose, 532; curare poisoning, 591; acetone bodies, 819
Voit, W., 814
Voitinovici, A., 106, 114, 121
Volhard, F., 469, 476, 506
Volhard, J., 759, 760
Volkmann, A. W., 899
Vorländer, D., 247
v. Vornveld, J. A., 341
Vossius, A., 441
Voswinckel, H., 843
Vozárik, A., 675, 677
de Vries, H., 2, 5, 6
Vulpian, A., 377, 441

Waage, P., 32
Wachsmann, M., 501
Wachsmuth, L., 356
Wäkchli, G., 117
de Waele, H., 653
Wagner, B., 813
Wagner, H., 572, 577
Wahlgren, V., 417, 420, 440, 838
Wait, Ch., 595
Wakemann, A. J., 386, 387, 737, 774
Walbum, L. E., 50, 793
Waldvogel, R., 820
Walker, J., 28
Wallace, G. B., 89
Walter, Fr., 682, 851, 856
Walter, G., 173, 630
v. Walther, P., 535, 537
Walton, J. H., 35
Wanach, R., 304
Wang, E., 730, 731
Wanklyn, J. A., 647
Wauner, Fr., 870
Warburg, O., leucine, 142, 143; fertilization, 640; phenylamino acetic acid, 786; oxydation processes, 873, 874
Warfield, L. M., 455
Warmbold, H., 647
Warren, J., 593
Wasbutzki, M., 519
Wassiliew, W., 499
Wasteneys, H., 73
Waterman, N., 379
Waymouth, Reid, E., 533
Weber, 635

Weber, O. H., 341, 555, 340
Weber, S., 594, 692, 693
Wechselmann, Ad., 733
Wechsler, E., 83, 117
Wedenski, N., 749
Wegrzynowski, L., 716
Wehrle, E., 404
Weidel, H., 189
Weigert, Fr., 163
Weigert, R., 265
Weil, Arth., 161, 605, 144
Weil, F. J., 423
Weiland, W., 786
Weinland, E., lactase, 53, 492, 532; glycogen, 390, 395; sugar formation, 412; retarding substances, 466, 487, 892; fat formation, 561;
Weintraud, W., 403, 404, 685, 761
Weis, Fr., 506
Weisbach, 613
Weiser, St., 264
Weisberger, G., 866
Weiske H., cellulose 510; bones, 554, 555; asparagin, nutritive value, 912
Weiss, F., 78, 83, 109, 111, 162
Weiss, H. R., 505, 506
Weiss, J., 721
Weiss, Sigm., 395, 592
Weisz, Mor., 741, 752
Weizmann, Ch., 87
Wellman, O., 896
Wells, H. G., keratin, 114; hypophysis, 380; liver nitrogen, 387; uric acid, 701–703, 706
Wendel, A., 143
v. Wendt, G., 882, 903
Wenz, R., 128
Wenzel, F., 178–180, 577
Werenskjold, F., 659
Werigo, B., 95, 866
Werncken, G., 651
Werner, A., 552
Wertheimer, E., 440, 498, 499
Werther, M., 459, 590, 591, 593
v. Westenrijk, N., 309
Wester, D. H., 839, 840
Wetzel, G., 122, 160, 178
Weyl, Th., albumin crystals, 94; carbonmonoxy methæmoglobin, 287; amniotic, fluid, 642, 643; creatinine, 695; benzoic acid, 723; nitrate, 766
Wheeler, H., L., iodogorgonic acid, 123; phenylalanine, 152; nucleic acid, 185; cytosine, 194
Whipple, G. H., 253, 442
White, B., 705
Withney, J. L., 406
Wichmann, A., 261, 652
Widdicombe, J. H., 492
Wichowski, W., urea determination, 688; uric acid, and allantoin, 705, 706, 716–718; hippuric acid, 721
Wieland, H., 423
Wiener, H., autolysis, 43; serumglobulins, 259, 261, 335, nucleic acids, 514; uric acid, 699, 700, 702, 704–706; oxalic acid, 716
Wiener, K., 182
Wihelmy, 33
Wilke, K., 298
Willcock, Ed., 634
Willdenow, C., 164
v. Willebrand, E. A., 849
Willheim, R., 103
Williams, D., 510
Williams, H. B., 827
Willstätter, R., proline 154; lecithin, 263; glycerinphosphoric acid, 247; chlorophyll and blood pigments, 277, 296, 297; cholesterin, 445, 448; carotein yolk lutein, xanthophyll, 631
Wilson, R. A., 606, 608
Wimmer, M., 914
Windaus, A., histidin, 159, methylimidazol, 201; cholesterin, 445, 448, 449
Windrath, H., 529
Winkler, 559
Winogradow, A., 416
Winteler, L., 416
Winter, J., 660
Winterberg, A., 334, 675, 683
Winternitz, Hugo, blood pigment determination, 302; amounts of hæmoglobin, 338; bile, 440; putrefaction, 517, 724; iodized fat, 560, 668, 669
Winternitz, M. C., 371, 702
Winterstein, E., amino-acids, 141, 153; arginine, 161; lysine, 163; hexone bases, 164; phosphatides, 245; phytin, 579; colostrum, 658; tunicin, 839; chitin, 839
Wislicenus, J., 596
Wittmaack, K., 663
Woeber, A., 126
Wöhler, Fr., hippuric acid synthesis, 39, 783; urea, 689; demolition of uric acid, 705, allantoin, 716, 717
Wörner, E., 607, 611, 711
Wohl, A., 200
Wohlgemuth, J., autolysis, 43; enzymes, 53, 71; oxyaminosuberic acid, 147; oxydiamino-sebacic acid, 165; pentoses, 203, 208, 210; diastase in blood, 298; ferratin, 383; glycogen, 393, 394; cystine demolition, 442; gastric juice, 476; pancreatic juice, 500; pancreatic diastase, 501; pancreatic rennin, 509; enzyme of egg-yolk, 628; woman's milk, 660, 661; conjugaged glucuronic acids, 751; amino acids in urine, 756
Wolf, C. G., glycogen, 397; urinary nitrogen, 680; creatinine, 693; cystinuria, 827, 828; urinary sulphur, 882
Wolfenstein, R., 737
v. Wolff, E., 595
Wolff, H., 219, 358, 841
Wolff, J., 873
Wolff, L. W., 19

Wolffberg, S., 395, 861, 864, 865
Wolkow, M., 735, 736
Woll, F. W., 645
Wolter, O., 770
Woltering, H., 340, 383
Woods, H. S., 246
Wooldridge, L. C., stroma, of blood corpuscles, 274; tissue fibrinogen, 306, 307; blood coagulation, 315, 321, 323, 324
Worm-Müller, J., blood, 339, 340, 343, 344; sugar test, 803; sugar determination, 811–813
Worms, W., 635
Woronzow, W. N., 381
Wright, A., fibrin ferment and coagulation, 256, 313, 323; blood alkalinity, 309; diabetes, 400
Wróblewski, A., fermentation, 41; pseudonuclein, 104; starch, 227; pepsin, 466, 470; enzyme action, 510; enzymes of brain, 606; milk, 660, 661
Wulff, C., 190, 714
Wurm, W. A., 843
Wurster, C., 768
Wurtz, A., 346, 625

Yagi, S., 448
Yakuwa, G., 912
Yoshimoto, 443
Yoshimura, K., 579
Young, P. A., 435
Young, R. A., 230, 391
Young, W. J., co-enzymes, 52, 205; carbohydrate-phosphoric acid ester, 60, 205; dioxyacetone, 205
Yvon, 752

Zängerle, M., 626
Zahór, H., 794
Zaitschek, A., milk, 651, 653, 658–662, 668
Zak, E., 354, 769, 791
Zaleski, J., leaf and blood pigments, 277, 296; blood pigments, 291–295, 301; ammonia, 334, 336, 528, 683, 768; urea, 684; liver and acid formation, 685, 703, 704
Zaleski, St., iron of liver, 383, 388; milk, 666, 667; reaction of intestinal contents 519
Zalesky, N., 552, 847
Zander, E., 839
Zanetti, C. U., seromucoid, 260, 263; bile, 417; ovomucoid, 635
Zangermeister, W., 302, 642
Zaribnicky, Fr., 348
Zaudy, 701
Zdarek, E., 841
v. Zebrowski, E., 453
Zeehuisen, H., 777
Zeidlitz, P. V., 804
Zegla, P., 398
Zeller, A., 758, 777
Zemplén, G., 839
v. Zeynek, R., dermoid-cyst-fat, 239, 627, 844; blood pigments, 283, 285, 289, 290; liver, 602; bile, 438; sarcomelanin 841; chromoproteid, 843
Zickgraf, G., 83, 870
Ziegler, E., 779
Ziegler, J., 32, 708, 716, 717
Zillessen, H., 593
de Zilwa, L., 500
Zimmermann, R., 372, 725, 726
Zimnitzki, S., 517
Zink, J., 237, 559
Zinnowsky, O., 277
Zinsser, A., 476
Zisterer, J., 917
Zobel, S., 581
Zoja, L., oxyproteic acids, 83; elastin, 122; ovalbumin, 633; urobilin, 744; uroerythrin, 748; hæmatoporphyrin, 797, 798
Zsigmondy, R., colloids, 15, 19, 21, 23
Zuelzer, G., lecithin, 245; diabetes, 405, 406; skin breathing, 849
v. Zumbusch, L., 434, 844
Zuntz, N., blood, 310, 338, 340, 341; glycogen, 391; sugar in blood, 398; phlorhizin diabetes, 400; digestion, 506, 542; protein assimilation, 525; muscle fat, 586, 596; muscle metabolism, 591, 595, 597; pig milk, 659; high altitudes, 341, 848; skin breathing, 849; gases of blood, 851–853, 859, 866, carbonic acid of lymph, 856; alveolar air, 863; respiration, 868, 869; metabolism, 879, 884, 890, 893, 922, 923, 926, 927, 929; calculation of calorific power, 888; nutritive value of albumoses, 912; alcohol, 921; digestion work, 930
Zuntz, L., 309
Zuntz, E., digestion products, 132–134, 139; gastric digestion, 482, 484; absorption, 484, 526, 532, 540; trypsinogen and its activation, 496, 497; intestinal digestion, 513; muscles ,572
Zweifel, P., 456, 500, 748, 749

GENERAL INDEX

Abderhalden and Schmidt's reaction for proteins, 101
Abiuret products, 132, 513, 526
Absorption, 524–543
  chemical processes during, 524–525
  effect of extirpation of the pancreas upon, 531, 532
  removing portions of intestine upon, 541, 542
  of amino acids from intestine, 526–528
  of bile constituents, 540, 541
  carbohydrates, degrees of rapidity of, 532–534
  of fat, 535–540
  of nonbiuret giving products from intestine, 526–528
  of mineral substances, 540
  peptones from intestine, 526–528
  of proteose from intestine, 526–528
  of undigested protein, 525, 526
  theory of, 542, 543
Acetanilide, fate of in organism, 779
Acethaemin, 292
Acetone bodies, 776
  in urine, 818–828
  origin of, 818, 819
  fate in organism, 821
  formation from fat, 820–822
  formation from protein, 819
  former, 781
  in urine, 822–824
  quantitative estimation of, in urine, 825
Acetophenone, fate of in organism, 785
Acetylation in organism, 786
Acetyldiglucosamine, 839
Acetyl equivalent of fats, 238
Acetylglucosamine, 784
Acetylene hæmoglobin, 288
Acetylpara-amidophenol, 780
Achilles tendon, 545
Acholia, pigmentary, 439
Achroodextrin, 229, 458
Acid abietinic, 447
  acetic, in intestines, 513
    in stomach, 465
    in urine, 748, 773
  acetoacetic in urine, 821, 824, 825
    fate in organism, 821
  acetylaminobenzoic, 784
  albuminates, 125–127
    absorption of, 533
    in peptic digestion, 472
  alloxyproteic, in urine, 752, 754, 755
  amides, behavior in organism, 775
  aminoacetic. See Glycocoll.
  aminocaproic. See Leucine, 141
  α-diamino-β-dithiolactic, 148
  α-γ-diaminovaleric,, 163
  α-ε-diaminocaproic, 163
  d-α-amino-*n*-caproic, 144
  aminoethylsulphonic, 150
  α-aminoglutaric, 146, 147
  α-aminoisobutylacetic. See Leucine.
  α-amino-valeric, 140. See Valine.
  aminobenzoic, behavior in organism, 783
  aminocinnamic, 778, 780
  α-amino-β-oxypropionic, 145. See Serum.
  α-amino-β-thiolactic, 148, 150. See Cystine,
  α-aminopropionic, 140. See Alanine,
  aminoglucuronic, 548
  aminohippuric, 783
  aminophenylacetic, behavior in organism, 781
  aminosuccinic. See Aspartic acid, 146
  antoxyproteic in urine, 752
  arachidic, 232, 627, 645, 845
  aspartic, 85, 146
    quantity in proteins, 106, 107, 115, 125
    relation to formation of urea, 682, 776
    relation to formation of uric acid, 702
  β-amino-α-oxypropionic. See Isoserine, 146
  β-imidazol-α-aminopropionic, 159

Acid benzoic, conjugation of, in organism, 782
in urine, 723
glucuronic, 751
benzoyl-amino acetic. See Hippuric acid, 719
bilinic, 429
bilianic, 423
bilirubinic, 430
bromphenylmercapturic, 780
butyric, fermentation, 214, 516
camphoglucuronic, 222, 751, 786
capric, 232
caproic, 232
caprylic, 232
carbamic, 267
conjugation of, 786
fate of in organism, 786
in blood, 267, 683
in urine, 691, 692
carbamino-acetic, 164, 856
carboglobulinic, 855
carbolic. See Phenol.
carminic, 844
carnaubic, 239, 673
carnic, 653
caseanic, 85, 165
caseinic, 85, 165
cephalic, 248
cepholinic, 248
cerebrinic, 612
cerebrinic-phosphoric, 612
cerebronic, 611
cerotic, 239
cheno-taurocolic, 422, 427
chitaminic, 219
chitaric, 219
chlorhodinic, 366
chlorphenylmercapturic, 786
cholalic, 423–425
constitution of, 423
cholanic, 425
choleic, 425
cholesterinic, 423
cholic, 423, 424
properties of, 424
tests for, 424
choloidanic, 423
choloidic, 427
cholylic, 423
chondroitic. See Chondroitinsulphuric acid, 547
chondroitin-sulphuric, 171, 173, 547
chrysophanic, elimination in urine, 787
cilianic, 423
cinnamic, behavior in organism, 719
citric, in milk, 647, 658, 663
coccinic, 844
cochenillic, 844
combined hydrochloric, 488
cresol-sulphuric, 724–727
crotonic, 825
Acid cumic, conjugation of in organism, 783
cuminuric, synthesis of in organism, 783
cyanuric, 687, 698
cysteinic, 149
damaluric, 758
damolic, 758
dehydrobillinic, 429
dehydrocholeic, 425
desaminoalbuminic, 127
desoxycholic, 425, 426
properties of, 426
diacetic. See Aceto-acetic
dialinic, relation to formation of urine, 704
diamino acetic, 85
diaminotrioxydodecanoic, 85, 165
dimethylaminobenzoglucuronic in urine, 751, 786
p-dimethylaminobenzoic, 786
dioxydiaminosuberic, 165
dioxyphenylacetic in urine, 735
dioxyphenyl-lactic, 738
dioxystearic, 236, 647
doeglic, 237
elaic, 236
elaidic, 236
ellagic, 524
equivalent, 238
erucic, 232
ethylidenelactic, 582
euxanthic, 222, 223
in urine, 785
euxanthonglucuronic, 222
excretolic, 523
fellic, 426
fermentation lactic, 582
in blood, 335
in coagulation of milk, 585, 645
in muscle, 604
in stomach, 487, 488
formic, fate in organism, 748, 773
furfuracrylic in urine, 784
furfuracryluric in urine, 784
gadoleic, 237
galactonic, 217
gallic, fate of in organism, 785
in urine, 734
gentisic, 336, 737
behavior in organism, 784
gluconic, 198
in diabetes, 403
glucosaminic, 201, 219, 221
in milk, 643
glucothionic, 387, 673
in milk, 643
glucuronic, 221–223
conjugation with, 785
elimination, effect of foreign substances on, 750

Acid glucuronic, formation of in organism, 751
in bile, 417, 433
in blood, 264
in urine, 750
isolation of, 222
preparation of, 223
properties of, 222
quantitative estimation of, 223
glutamic, 82, 86, 106, 107, 115, 125, 147.
glutinic, 119
glyceric, 145
glycerophosphoric, 248
in urine, 749, 757
glychollic, fate of in organism, 713
glycocholic, 417, 419, 420
absorption of, 548
in feces, 517
preparation of, 421
properties of, 420
glycocholeic, 420–421
preparation of, 421
properties of, 420
glycosuric in urine, 735
glycuronic, 221
glyoxylic, 719
as reagent, 100
guanidineacetic, fate of in organism. See Glycosamine, 787
δ-guanido-α-aminovaleric. See Arginine, 161
guano bile, 421
guanylic, 178, 181, 183, 184
of liver, 383
hæmatinic, 296, 430
hæmatopyrrolidinic, 291
hæmoglobin, 285
hippuric, 267, 719–723
formation of, 139
in organism, 719
in urine of herbivora, 720, 721
occurrence of, 719 720
preparation of, 722–723
properties of, 722
quantitative estimation of, 723
reactions for, 722
synthesis of in organism, 782
synthetical preparation of, 719
theories of formation in organism, 721
homogentisic, 778
formation of in organism, 735–737
in urine, 727, 734–740
mother substances of, 735–736
preparation of, 739
Acid homogentisic, properties of, 738–739
quantitative estimation of, 739
quantity eliminated, 735
test for, 739
homoglytisic, origin of, 736
hydrochloric action upon ptyalin, 455
secretion of bile, 416
secretion of pancreatic juice, 498
pylorus, 480, 482
antifermentive action, 485
hydrochinon-sulphuric, 724
hydrocinnamic, behavior in animal body, 720
hydrocyanic, effect on blood pigments, 285
pepsin digestion, 471
trypsin digestion, 506
hydroparacoumaric, in intestinal putrefaction, 515
in urine, 734
hydroquinone carboxylic, 738
hydroquinonesulphuric, 691, 724, 728
hyoglycocholic, 421, 427
hypogaeic, 239
imidazolaminoacetic in urine, 758
imidazol propionic, 82
indolacetic, 82
in urine, 733
indolaminopropionic, 82, 155
indol-carboxylic, 728
indol-propionic, 82, 155
indoxyl-carboxylic, 728
indoxylglucuronic, 728
indoxyl-sulphuric, 724
formation of in organism, 728
in urine, 728–731
inosinic, 178, 181–183, 185
iodogorgonic, 123
isobilianic, 423
isocholanic, 425
isocholic, 427
isophonopyrrol carboxylic, 429, 430
isovaleric, 774
jecoleic, 237
kynurenic, 740, 758
in urine, 734
kyroproteic, 83
lactic, 145
formation of in active muscles, 593
in blood, 333
in blood, 334
in cerebrospinal fluid, 360

Acid lactic, in relation to diabetes, 408
origin of, 583, 584
quantitative determination in gastric contents, 488
test for in gastric contents, 488
transformation into uric acid, 704
lactophosphocarnic, 645, 653
lanoceric, 846
lanopalmitic, 846
lauric, 232, 647, 846
lepidotic, 844
leucinic, 142
levulinic, 211, 654
linolenic, 232
linolic, 232
lithobilic, 524
lithocholic, 427
lithofellic, 427, 524
lithuric, 758
lysalbinic, 127
lysuric, 164
maleic, 290
malic, fate in organism, 668, 676
mandelic, 780
mannonic, 212
margaric, 235
melanoidic, 841
menthol-glucuronic, 817
mesitylenic, conjugation of in organism, 783
mesitylenuric synthesis of in organism, 783
metaphosphoric as precipitant of proteids, 98, 789
in nucleins, 176
in pseudonucleins, 177
methylethyl-α-aminopropionic, 143. See Isoleucine.
methylguanidine-acetic. See Creatine, 573
methylhydantoic, 776
monoxystearic, 233, 236
mucic, 217, 394, 654
muconic, 778
myristic, 232
naphtholglucuronic, 787
neurostearic, 611
nitrobenzoic, 784
nitrohippuric, 783
nitrophenolpropiolic, fate in organism, 728, 731
test for dextrose, 216, 808
norisosaccharic, 219, 630
nucleic, 622
of milk, 643
yeast, 178, 185
nucleotinic, 179
nucleotinphosphoric, 179
oleic, 232
properties of, 236
tests for, 237
Acid ornithuric, 163
synthesis of in organism, 783
orotic, 654
orylic, 653
oxalic in urine, 716, 717
oxaluric, 699, 715
oxaminic, 83
oxonic, 699
oxyaminosuberic, 85, 148
oxyaminosuccinic, 85, 147
oxy-α-pyrolidinecarboxylic. See oxyproline.
oxybenzoic, fate in organism, 783
β-oxybutyric, 821, 825–827
detection of in urine, 826
fate in organism, 821
formation from amino acids, 821
properties of, 825, 826
quantitative estimation of, in urine 826
oxydiaminosebacic, 85, 165
oxydiaminosuberic, 85, 164
oxyhydroparacoumaric, in urine, 734
oxyisovaleric, 774
oxymandelic, 739
oxymethylpyrazine-carboxylic, 221
p-oxyphenylacetic, 515
in urine, 734, 784
p-oxyphenyl-α-aminopropionic. See Tyrosine, 152
p-oxyphenylpropionic, 515, 734
in urine, 784
oxyphenylpyroracemic, 737
oxyproteic in urine, 752, 754
oxyprotosulphonic acid, 83
oxyquinoline carboxylic. See Kynurenic acid.
palmitic, 232, 235
properties of, 236
parabanic, 699
paralactic, 582
origin of, 583, 584
in urine, 749
paranucleic, 652
paraoxyphenyl-acetic in urine, 734, 735
paraoxyphenylpropionic, 82
in urine, 734, 735
pepsin-hydrochloric, 473
peroxyproteic, 83
phenaceturic in urine, 723
phenol glucuronic, 222, 725, 749, 751
phenol-sulphuric, 724, 725–727
phenylacetic, 82
conjugation of in organism, 783
phenylaminoacetic, 780
phenyl-α-aminopropionic. See Phenylalanine, 151
phenylbutyric, 781

Acid phenylcaproic, 781
phenylketopropionic, 781
phenyllactic, 736, 778, 780
phenyl oxypropionic, 781
phenylpropionic, 82
phenyl pyroracemic, 737
phenylvaleric, 781
in perspiration, 848
in urine, 724
phonopyrrolcarboxylic, 298
phosphocarnic, 267, 572, 577–579
in active muscles, 594
in urine, 757
source of muscular energy, 598
as nuclein amounts in blood of different animals, 326
in urine, quantitative estimation of, 763–764
phthalic, fate in organism, 779
physetoleic, 239
plasminic, 186
polypeptide phosphoric, 652
protalbinic, 127
protic, 572
protocatechuic, fate in organism, 726
pseudonucleic. See Paranucleic acid
psyllic, 845
pulmotartaric, 870
pyinic, 366
pyridine-carboxylic, fate of in organism, 784
pyridinuric, 784
pyrocatechin-sulphuric, 724
in urine, 727
pyrocholoidanic, 424
α-pyrolidine carboxylic. See Proline, 154
pyromucic in urine, 784
pyromucinornithuric in urine, 784
pyromucuric, 784
pyroracemic, 150
effect of yeast upon, 206
pyrrolidone-carboxylic. See α-Proline, 113
pyruvic, effect of yeast upon, 206
quinic, 719
racemic, fate in organism, 773
renosulphuric, 673
rhodizonic, 580
saccharic, 198, 222
behavior in diabetics, 403
relation to glycogen formation, 394
salicylic, conjugation of in organism-783
salmo-nucleic, 178
sarcolactic, 582
in brain, 606
in lymphatic glands, 366
Acid sarcolactic, in muscular work and rigor, 591–595
in the bones, 556
passage into urine, 748
sarcomelanic, 841
scymnol, 417
scymnol-sulphuric, 417
sebacic, 236
silicic, in blood, 268
in bones, 555
in connective tissue, 549
in feathers and hair, 839
in hen's eggs, 632, 637, 639
in urine, 769
skatol acetic, 82, 156
skatolaminoacetic, 156
skatol-carboxylic in urine, 733
skatoxylglucuronic, 222, 732, 750
skatoxyl-sulphuric, 724, 732, 733
origin of, 732
stearic, 232
properties of, 235
succinic, 83
in fermentation of milk, 645
in intestines, 513
in perspiration, 848
in spleen, 370
in thyroid, 373
in transudates, 355, 359, 361
in urine, 749, 773
sulphosalicylic as a reagent, 790
sulphuric, action on peptic digestion, 469. See also Ethereal sulphates and Mineral substances.
tannic, fate of in organism, 785
tartaric, relation to glycogen formation, 394
in organism, 773
in perspiration, 848
tartronic, 704
taurocarbamic, 776
taurocholic, 417, 421–422
preparation of, 421, 422
properties of, 422
taurocholeic, 422, 423
tetraoxyaminocaproic, 548
therapinic, 237
thiolactic, 150
thiophenuric in urine, 784
thymic, 179
thymonucleic, groups, 181
thiolactic, 85
toluic, conjugation of in organism, 783
toluric, synthesis of in organism, 783
trichlorethylglucuronic. See Urochloralic acid.
triticonucleic, 178, 185
2-, 4-, 5-trimethylpyrrol-3-propionic, 429
trioxybenzoic. See Gallic.
turpentine glucuronic, 785, 817
tyrosinesulphuric, 153
uraminobenzoic, 783

Acid uramin-salicylic, 783
urea glucuronic, 750
uric, 187, 267
amounts formed in organism, 707
as a pigment, 844
effect of food on elimination of, 700
endogenous origin, 702
exogenous origin, 702
fate of in organism, 705, 706
formation of from purines, 701, 702
in birds, 703, 704
of in the organism, 701–707
from lactic acid, 704
in blood, 334
in muscles, 572
in urinary sediment, 830
in urine, 698–712
occurrence of, 699, 700
preparation from urine, 710
preparation of, 698
properties of, 698–699, 707, 708
quantitative estimation of in urine, 710
relation to urea elimination, 701
quantity in various urine, 699, 700
synthetic formation of, in organism, 705
tests for, 709
various factors effecting elimination, 700–702
urocanic, 758
urochloralic, 777
uroferric in urine, 752, 755
uroleucic in urine, 736, 739
uronitrotoluolic in urine, 786
uroproteic in urine, 754
uroxanic, 699
ursalicylic in urine, 783
ursocholeic, 427
valeric, 80, 142
vanillinic, 780
whey, 645
xanthobilirubinic, 429
xanthopyrrolcarboxylic, 299
yeast-nucleic, 179, 185
Acids, amino, 92
aromatic, fate of in organism, 780–783
investigations on, of demolition in organism, 780–783
as acetone formers, 819
as carbon dioxide binders, 855, 856
conjugation with, 786
deamidation, 682
fate of, in organism, 774–776
Acids, amino, formation in tryptic digestion, 507
of, in liver, 530
how absorbed, 528
in globin of blood pigments, 289
in homogentisic acid formation, 736–738
in liver tissue, 383
in lymphatic glands, 366
in muscles, 572
in transudates and exudates, 355
in serum, 267
in urine, 756, 827
investigation on the demolition of, in organism, 774–776
of pseudomucin, 626
Sörensen's, formol titration for, 139–167
synthesis of, in organism, 786
sugar formation from, in liver, 412
transformation of, into urea, in the organism, 683, 684
aromatic, fate of, in organism, 784
-oxy, in urine, 733, 734
bile, 267
detection of, 418, 419, 427, 428
biliary, properties of alkali salts, 418
caseonphosphoric, 652
cholic, preparations of, 426
conjugated glucuronic in urine, 817–818
desaminoproteic, 83
diamino, 84, 159–163
ethereal sulphuric, 723–733
amounts in urine, 724
in urine, 765
quantitative estimation of, 726
synthesis in liver, 381
excitants for bile secretion, 416
fatty, 232–239, 265
amounts in blood of different animals, 328
in brain, 605
in blood, 334
in perspiration, 848
in pus, 365, 366
in urine, 748, 773, 829
investigation on the demolition of, in organism, 773, 774
series, fate of, in organism, 773, 774
glucuronic, conjugated, 222

Acids, glycocholic, preparation of, 421
in large intestine, from putrefaction, 515
in spleen, 370
in thymus, 368
in thyroid gland, 373
in transudates and exudates, 355
kyroproteic, 83
lactic, 582–586
detection of, 585
in bones, 556
in urine, 703, 704, 748
mercaptic, elimination of, 786
properties of, 585
melanoidic, 841
mineral, alkali-removing action of, and action on the elimination of ammonia, 675, 676, 762, 768, 856 893
monoamino. See Acids, Amino.
neutralization of, in the organism, 675, 676
nucleic, 175, 177–186
complex, 181
effect of gastric juice on, 473
enzymotic, cleavage of, 508
plant, 185–186
simple, 181
organic, behavior in animal body, 767, 772, 773, 776, 777
oxyamino, 79, 80, 84, 148, 219
oxyfatty, in animal fat, 232, 238
oxy, in urine, detection of, 735
oxypropionic, 582
oxyproteic in urine, quantitative estimation of, 755, 756
phthalic, fate of, in the organism, 778
proteic in blood, 267
in serum, 267
in urine, 752, 754
thymonucleic, 178
thymus-nucleic, 173
uramino, 786
ureido glucuronic, 750
volatile fatty, in urine, 748
Acidosis, 820, 821
Acrite, 212
Acrolein, 234, 237
α-acrose, 212
β-acrose, 212
Actiniochrom, 844
Activators, 60
Adamkiewicz-Hopkin's reaction for tryptophane, 157
Adamkiewicz's reaction for protein, 100
Adaptation of the glands, 454, 462, 463, 494–496
Addison's disease, 377, 379
Adelomorphic cells, 489
Adenase, 48, 188
Adenine, 178, 181, 185, 187, 188, 192, 193, 712
Adenine-hexose compound, 180
Adenosine, 180
Adialyzable bodies in urine, 757
Adipocere, 560
Admissibility, theories of, 9
Adrenalin, 378–380
bodies, 377, 378
constitution of, 378
function of, 379, 380
properties of, 379
tests for, 379
"Adsorpates," 12
Adsorption, 49, 62, 63, 69
in relation to permeability, 11, 12
Ægagrophilæ, 524
Ærotonometric method, 864
Age, effect upon metabolism, 922, 924
Agglutenins, 47, 69
Agmatine, 162
Air bladder, of fishes, gases of, 867
Alanine, 85, 106, 107, 109, 111, 113, 125, 140, 145
Alanylalanine, 88
Alanylalanineglycin, 86
Alanylglycine, 87, 508
Alanylleucine, 86, 508
Albamine, 220
Albumin, 49
detection of, in urine, 791
Albuminates, 104, 125–127
acid, 92, 126
alkali, 91, 92, 125, 126, 533
Albuminoids, 92, 112
Albuminose, 623
Albumins, 91, 92, 93, 102, 103, 106
quantitative estimation of, in urine, 793
properties of, 102, 103
Albumoids, preparation of, 549
Albuminuria, 771, 787
Albuminoids, 92
Albuminous bodies, in general, 94–97
Albumoid, properties of, 618
Albumose, alkali, 127
Albumoses, 130
Alcapton in urine, 727
Alcaptonuria, 735–740
Alcohol, aminoethyl, 240
cetyl, 239
melissyl, 239
myricyl, 239
Alcoholase, 47, 48
Alcoholases, 875
Alcoholic fermentation, 41
Aldehydases, 875
Aldehydes, fate of, in organism, 775, 780, 781
Aldoses, 197
Alexines, 266
Alimentary acetonuria, 820
glycosuria, 533
Alizarin, elimination in urine, 556, 787
Alkali earths. See Mineral substances.

Alkaloids, fate of, in organism, 787
Alkyl sulphides, 846
Allantoin, 699, 717–719
elimination of during poisoning, 717, 718
formation of, in organism, 717
occurrence of, 717
preparation of, 718
properties of, 718
tests for, 718
Alloisoleucine, 145
Alloxan, 86
Alloxuric bases, 186, 712
preparation of, 714. See Purines.
Alveolar air, 853
Ambergris, 524
Amboceptors, 69
Ambrain, 524
Amicrons, 19
Amidases, 702
Amidoguanine, 718
Amidomyelin, 609
Amidulin, 227
Amino acids. See Acids, Amino.
Amino aldehyde, 219
butyrobetaine in urine, 787
cerebrinic acid, glucoside, 612
oxypurine. See Guanine.
oxypyrimidine. See Cytosine.
sugars, 219–221
Aminophenol, 779
Aminopurine, 191
Aminosuccinic-acid amide. See Asparagine
Ammonia, amounts in urine, 766
detection of, in urine, 768
elimination in relation to acid formation, 766, 767
importance of to organism, 767
in blood, 334
in urine, 766–768
in venous blood, 336
quantitative estimation of, in urine, 768
transformation of, in the liver, 767
Ammonium magnesium phosphate, in intestinal calculi, 232
in urinary calculi, 834
salts, relation to glycogen formation, 394
relation to urea formation, 683, 767
relation to uric acid formation, 702
urate, in urinary calculi, 834
in urinary sediment, 829
Amniotic fluid, 642
Amphicreatine, 578
Amygdalase, 48
Amygdalin, cleavage of, 59
Amylase, 47, 48
Amylodextrin, 227, 229
Amyloid, 172, 231
preparation of, 174
Amylopectin, 227
Amylopsin, 501
Amylose, 227
soluble, 227
Amylum. See Starch, 226
Anaphylaxis, 70
Aniline, fate of in organism, 779
Animal oxidation, 42
gum in urine, 749
Anions, 5
Anisotropous muscle substance, 565
Antedonin, 844
Antialbumate, 472
Antialbumid, 472
Antibodies, 66, 68
Antidonin, 844
Antienzyme, 63
Antigens, 66, 68, 69
Antiketoplastic action, 820
Antimony, action on N-elimination, 679
passage into milk, 671. See Mineral substances.
Antipepsin. See Enzymes, 464
Antipeptone, 130
Antipyrine, fate of, in organism, 785
Antirennin, 69, 474
Antithrombin, 322
Antitoxins, 47, 66
Antitrypsins, 503
Anuria in cholera, 848
Aorta elastin, 116
Apatite in bone ash, 553
Amphopeptone, 129
Aporrhegmas, 167
Aqueous humor, 361
Arabinoses, 198, 210. Structural formulæ for, 198
d-arabinosinine, 201
Arabite, 198
Arachnoidal fluid, 355
Arbacin, 108
Arbutin, 394, 727
Arginase, 48, 91, 161
Arginine, 85, 106, 107, 108, 109, 111, 113, 115, 117, 119, 125, 161, 162
in various proteins, 165
cleavage into creatine, 575
Arginine-histone peptone, 139
Argon in blood, 850. See also Gases
Arnold and Lipliawsky's reaction for acetoacetic acid in urine, 824
Arnold's reaction for proteins, 100
urine reaction, 696
Aromatic combinations, fate in organism, 776–784
Arsenic poisoning, 439–441
on nitrogen elimination, 679
Arterin, 276
Ascitic fluid, 357
Asparagine, 146
Asparagus, effect on odor of urine, 764

Assimilation limit, 533
Atheromatous cysts, 142
Atmidalbumin, 130
Atmidalbumose, 130
Atmidkeratin, 113
Atmidkeratose, 113
Atropine, effect on uric acid elimination, 700
Auto digestion, 43
Auto-oxidation,
Autolysis, 43
as a protective agent, 46
effect of arsenic upon, 44
CO_2 on, 44
in organic colloids, on 44
oxygen upon, 44
radium on, 44
reaction upon, 43
importance of enzymes on, 45
various processes in, 44
Autolytic processes in life, 45
Autotoxines, influence on putrefaction, 529

Bacteria, influence on putrefaction, 520
Bacterial action in intestinal canal, 518
Bacteriolysins, 69
Bacterium ureæ, 829
Balsam of copaiba, fate of, in organism, 787
Bang's method of estimating sugar, 809
Barium, 22
Basal requirement, 897, 922
Basedow's disease, 375
Baumann's test for dextrose, 216
Beer vinegar bacteria, enzymes of, 10
Beeswax, 239
Bela v. Bitto's reaction for acetone, 823
Bence-Jones' protein, 792
Benzaldehyde, 36
Benzene homologues, fate of, in organism, 779
ring, theory for splitting of, in organism, 778
Benzidine blood test, 798
Benzoylation of carbohydrates, 215, 216, 749, 808
Benzoylchloride test for dextrose, 215
Benzoyl cystine, 150
Betaine, 246, 572
Bezoar stone, oriental, 426, 532
Bifurcated air, 853
Bile-acids, 419–428
detection of, 418, 419
in animal fluids, 427, 428
formation of, 442
in blood, 267, 333
in urine, 799–800
origin of, 440
relation to bile pigments, 444
Bile, 413, 442
absorption of, from the liver, 444
amounts secreted, 414–445

Bile, chemical formation of, 440–444
coloring matters, detection of, in blood, 435
composition of, in disease, 439
concretions, cholesterin stones, 445
pigment stones, 445
constituents, absorption of, 540, 541
constituents of, 417, 435
effect of in absorption of fats, 536–540
effect on putrefaction, 518, 519
gases of, 857
Hüfner's, 419
human, 437
composition of, 438
pigments, in, 438
properties of, 437
importance in absorption of fat, 511
mucus, 415, 437
passage into blood, 444
urine, 444
phosphorized constituents of, 439
properties of, 416, 417
putrefaction of, in intestine, 516
quantitative composition of, 436, 437
pigments, 427
formation of, 440–442
in feces, 522
in urine, 800, 801
origin of, 440, 441
relation to bile acids, 444
relation to blood pigments, 430, 442, 443
relation to urinary pigments, 743. 744
test for, 432, 433
salts, 417–419
properties of, 418
tests for, 418, 419
secretion, cholagogues, 415
effect of therapeutic agents on, 415
Biliary fistula, 413, 421
Bilicyanin, 435
Bilifulvin, 427
Bilifuscin, 434
Bilihumin, 435
Biliphæin, 427
Biliprasin, 434
Bilipurpurin, 435
Bilirubin, 427, 431, 516
derivatives of, 428–430
detection of, in blood, 435
Ehrlich's test for, 429
Hedenin's test for, 433
hemi-, 429
Huppert's test for, 429
hydro-, 428
of blood serum, 268
preparation of, 433
properties of, 430–432
quantitative estimation of, 433
Biliverdin, 433, 434
in excrements, 530

Biliverdin in feces, 530
properties, 434
preparation of, 434
Biological equivalence, 917
protein reactions, 266, 533
Bismuth, passage into milk, 670
Bitter substances, effect upon secretion, 461, 482
Biuret, 86
base, 86
reaction for proteins, 101
Blister-fluid, 362
Blood, 308–344
acid lactic, in, 334
alkalies in, 310, 311
ammonia in, 334
analyses of blood from various animals, 328
arterial, 335, 336
quantity of carbon dioxide in, 851
quantity of oxygen in, 851
at different periods of life,,
"buffy coat," 312
casts in urine, 796
clot, 251
coagulation, accelerating substances, in cell, 315, 316
calcium salts in, 316, 317
lime salts in, 316
methods of retarding, 312
prevention of, 251
retarding substances in, 312–326
theories for, 317, 321–323
changes in viscosity, 311
coloring matters, in urine. See also Blood pigments, 795–799
relation to bile pigments, 442, 443
corpuscles, 250, 272–305–308
constituents of, 274
determination of volume in blood, 326, 327
effect of water on, 6
salts on, 6
experiments with, 6
hæmagglutination, 275
hæmoglobin, 274, 276
hæmolysis of, 273
in lymph, 346
isolation of, 274
mineral bodies in, 304
number of, 272
size of, 272
non-nucleated, 250
Blood-corpuscles, nucleated, 250
protection of salts upon, 6
quantitative constitution of, 304
quantitative determination of, 326, 327, 328
red, osmotic phenomena with, 8
stromata, 274
stroma-fibrin, 275
stroma, 272, 273, 274
white, 250, 305–308
number of, 305
constituents of, 306–308
defibrinated, 252
degree of dissociation in, 271
determination of reaction, 271, 272
form elements of, 272–276
detection of, in urine, 796
determination of H ions in, 310
during pregnancy, 337
effect of alkalinity on carbon-dioxide content, 856
electrical conductivity, cleavages in,
enzymes in, 53, 332
fat in, 334
fatty acids in, 334
from muscular veins, 337
from veins of glands, 337
gas exchange in, 858–870
gases of, 857
gas tension in, 859–868
hepatic vein, from the, 336
human, analysis of, 328
importance of hæmoglobin in oxygen-carbon dioxide exchange in, 853
influence of food on, 338
injection of, 343, 344
in urine, 795–799
laky, 312
leucæmic, properties of, 342
constituents of, 342
leucocytes, increase in number of, 342
manner of binding of carbon dioxide in, 853, 854
menstrual, 337
mineral substances in, 335
non-coagubility of circulating, theories for, 319, 320
of various animals, analysis of, 328
of the two sexes, 337
of woman, analysis of, 328
oxidation in, 858
pigment arterin, 276
pigments, 276–305
acid hæmatinic, 296
acid hæmoglobin, 286
carbohæmoglobin, 288

Blood pigments, carbon dioxide-hæmoglobin, 288
carbon monoxide-hæmoglobin, 286, 287
carbon-monoxide methæmoglobin, 287
chlorocruorin, 303
cryptopyrrol, 297, 299
cyanhæmoglobin, 285
cyanmethæmoglobin, 285
decomposition, products of, 288, 289
detection of, 286, 287
formation of, 286
globin, 289
properties of, 289
hæmatin, 277, 290–292
formula for, 291
preparation of, 291
properties of, 291
spectroscopic action of, 292
hæmatin, reduced, 289
hæmatinogen, 300
hæmatocrystallin, 278
hæmatoglobulin, 278
hæmatoidin, 301
relation to bile pigments, 301
hæmatoporphyrin, 277
hæmatoporphyrin, spectroscopic examination of, 300
hæmatoporphyrin, preparation of, 300
hæmatoporphyrin, production of, 294–301
hæmatoporphyrin, formula for, 294
hæmatoporphyrin, relation to hæmatin, 294
hæmatoporphyrin, relation to bile pigments, 295
hæmatoporphyrin, behavior in animal body, 295
hæmatoporphryin, relation to plant pigments, 296
hæmin, 292–294
formula for, 292
properties of, 293
preparation of, 293
hæmachromogen, 289, 290
hæmerythrin, 303
hæmin crystals, 292
preparation, of, 293
Blood pigments, hæmochrom, 276
hæmocyanin, 303
hæmoglobin, 274, 276
hæmoglobin, composition of, 277
hæmoglobin, molecular weight of, 278
hæmoglobin, gas combining ability, 280
hæmoglobin, preparation from oxyhæmoglobin, 283
hæmoglobin, quantitative determination of, 301
hæmoglobin, Hoppe-Seyler's colorimetric method, 301
hæmoglobin, reduced, 282
hæmoglobin, spectroscopic quantitative method for, 302
hæmopyrrol, 297, 299
hæmorrhodin, 288
hæmochromogen, 276
hæmoverdin, 288
in urine, 795–799
isohæmopyrrol, 297
kathæmoglobin, 288
mesoporphyrin, 294, 300
methæmoglobin, 283–285
methæmoglobin, property of, 284, 285
methæmoglobin, preparation of, 285
nitric oxide-hæmoglobin, 288
neutral hæmatin, 288
phlebin, 276
phonoporphyrin, 295
phyllohæmin, 296
phyllopyrrol, 298, 299
phylloporphyrin, 296
porphyrinogen, 295
oxyhæmatin, 290
oxyhæmocyanin, 303
oxyhæmoglobin, properties of, 280, 281
parahæmoglobin, 281
parahæmoglobin, properties of, 281
photomethæmoglobin, 285
purple cruorin, 282
quantitative estimation of, 301–304
sulphhæmoglobin, 287
sulphur methæmoglobin, 287
tetroneryth:rin, 303

Blood plasma, 250, 252–264
analysis of, 269
plates, 250, 308
in coagulation, 314
poikillocytosis, 342
portal vein, from the, 336
quantitative composition of, 326–335
estimation of urea in, 691
quantity of, 343
bleeding of, 343
in organs, 344
reaction of, 76, 309–311
red corpuscles, decrease in number of, 341
effect of hemorrhage on, 341
effect of transfusion, 340
effect of transudation from, 340
effect of pressure on, 340
increase of, 340
increase of, theories for, 341
refraction coefficient of serum, 311
rennin acting enzyme in, 474
serum, acids in, 267
action of, on starch, 226
analyses of, 269
constituents of, 264–269
properties of, 264
rest in nitrogen, 267
pigments of, 268
quantitative analysis of mineral bodies, 270, 271
specific gravity, determination of, 309
spleenic vein, from the, 336, 337
"sucre virtual," 331
"sucre immediat," 331
urea in, 333, 334
uric acid in, 334
vascular regions, composition of, 335–344
venous, 335, 336
quantity of carbon dioxide in, 851
quantity of oxygen in, 851
Blueberry, pigments of, in urine, 787
Blue milk, 671
Blue stentorin, 844
Boar sperms, 622
Boas' test, for lactic acid, in gastric juice, 487
for HCL, 486, 487
Body, relation of weight and age of, to absolute consumption of material, 913
weight decrease during starvation, 885
Boiling-point, elevation of by colloids, 17
Bombicesterin, 449
Bondi-Schwarz's test for acetoacetic acid, 824
Bone, 551–558
ash, analysis of, 553
at different ages, 555
catabolism in starvation, 896
components of, 551
earth, analysis of, 552
effect of food upon, 556
marrow, 554
rachitic, 557
rachitis of, 555
softening of, 555
Bonellin, 844
Bony structure, matrix of, 551
Borneol, fate of, in organism, 785
Böttcher's spermine crystals, 621
Böttger-Almén's test, 215, 802
"Bowman's disks," 566
Boyle-Marriot's law, 28
Bradoxidizable substances, 4
Brain, constituents of, 604–606
epileptic, analysis of, 613
gray and white substance, compared, 605
human, analysis of, 612–613
paralytics, analysis of, 613
phosphatides of, 606–609
quantitative composition of, 612
British gum, 229
Bromhæmin, 294
Bromides, in saliva, 459
relation to formation of gastric juice, 477
Bromine, 22, 123
Bromoform, behavior in animal body, 775
Bromthymine, 195
Bromtoluene, behavior in animal body, 775
Brownian molecular motion, 20
Brücke's quantitative method for pepsin, 469
Buccal mucus, 453
"Buffy coat," 312
Bufidin, 846
Bufonin, 846
Bufotalin, 846
Bufotenin, 846
Bufotin, 846
Bunge and Schmeideberg, quantitative method for hippuric acid, 723
Burbot, sperms of, 181
Bursæ mucosæ, 361
Butalanine, 495
Butter, 647
Butterflies, pigments of, 844
Butylmercaptan, 844
Butyrinase in blood, 266
Butyrine, mono enzymotic synthesis of, 60
Byssus, 92, 122, 123

Cadaver alkaloids, 82
Cadaverine, 47, 82, 164

Cadaverine in urine, 827
Cæcum, 514
Caffeine, 187
Calcium carbonate in urinary sediment, 831
importance to enzymotic processes, 255, 256, 307, 312, 313, 498, 649, 650
in urine, 768(769
manner of excretion, 769
oxalate in urinary sediment, 830
phosphate in urinary sediment, 831
salts in blood coagulation, 308, 309
sulphate in urinary sediments, 831. See also Mineral substances.
Calculi-ammonium urate, 833
-calcium carbonate, 834
oxalate, 833
-cystin, 834
-fibrin, 834
Heller's scheme for investigating, 833
hemp seed, 833
intestinal, 531
-mulberry, 833
pancreatic, 509
-phosphate, 833
salivary, 459
-uric acid, 833
urinary, 828–829, 832–836
urinary, scheme for chemical analysis of, 836
urinary, chemical investigation of, 834–836
-urostealith, 834
-xanthine, 834
Caliphora larvæ, fat formation, 561
Calomel, effect upon excrement, 529
Calorific coefficients, 888
value of fat, 888
value of proteins, 888
milk protein, 888
starch, 887
urine quotient, 884
Cammidge's reaction for sugar, 815
Camphors, fate of, in organism, 000
Cane-sugar, 49, 224. See Sucrose.
Canirine, 578
d-Caprine, 144
Capronica's test for guanine, 191
Capsule of crystalline lens, 170, 617
Caramel, 214, 225
Carbamino reaction, Siegfried's, 166
Carbazole, fate of, in organism, 779
Carbohæmoglobin, 287
Carbohydrate (phosphatized), 2441
Carbohydrates, absorption of, 532–534
as a source of muscular energy, 598
benzoyl esters of, 202
classification of, 197
Carbohydrates, cyanhydrin synthesis for, 200
effect of gastric juice on, 473
ether-like combinations, 202
fate of, in organism, 885
fermentation of, 203–207
hydrazones, 202–203
in urine, 749–752
in venous blood, 336
osazones, 202–203
phosphoric acid esters of, 204–205
relation to histidine and purines, 202
See Various sugars.
Carbolic urines, 728
Carbon dioxide, acids, amino as binders of, 855, 856
effect of alkalinity on content in blood, 856
formation, calculation of, 889
hæmoglobin, 287, 853
as a binder of, 853
influencing oxygen absorption, 859
manner of binding in blood, 853–854
mechanism of elimination, 852–853
physical explanation of the giving up of, 866
proteins as binders of, 855
quantity in arterial blood, 851
venous blood 851
tension, 865
tension in tissue, 868
Carbon, elimination in organism, 883–884
monoxide blood, test for, 286
hæmochromogen, 288
hæmoglobin, 279, 285, 288, 300
methæmoglobin, 286
poisoning, 285, 402, 582, 679
Carboxylase, 206
Carnaubon, 240, 673
Carniferrine, 578
Carnine, 572, 577, 712
Carnitine, 572, 577
Carnomuscarine, 578
Carnosine, 572, 576–577
Carotin, 631
Cartilage, 546–550
components of, 540
constituents of, 549–550
gelatin, 549
hyalin, action of trypsin upon, 508

Caseid, 649
Casein, 49, 84, 91, 106, 647–652
cleavage products, of, 106
coagulation of, 649
a two-faced process, 650
theory, of, 650, 651
composition of, 647
properties of, 647, 648
hexone bases in, 165
human, preparation of, 652
of woman's milk, 661
peptic digestion of, 651, 652
preparation of, 652
solutions of, properties of, 649
Caseinates, 648, 649
Caseinokyrin, 136
Casecses, 130
Castoreum, 846
Castor lipase, 234
Castorin, 846
Catabolism of protein, 907, 908, 909, 911
Catalases, 43, 872
definition of, 33
equilibrium constant of, 33
in heterogeneous systems, 36
mass action on, 32
measurement of, 32
of benzaldehyde, 36
of diaceton alcohol, 35
of diazoacetic ether, 34
of esters, 32
of hydrogen peroxide, 35
reaction velocity of, 32
velocity coefficient, 34
function of, 7
Catalysts, 35
Catalytic processes, compared (organic and inorganic), 37
Cataphoresis, 20, 50
Cataract of lens, 619
Cations, 5
Cell-globulin, 274
-membrane, animal effect of gastric juice on, 473
of plant, effect of gastric juice on, 473
Cells, adelomorphic, 461
boundary layer of, 21
cover, 461
delomorphic, 461
lymphoid, 300
mineral constituents of, 22
pepsin, 461
permeability of, 34
rennin, 461
Cellobiose, 231
Cellose, 231
Cellulose, 231
fermentation of, in the intestine, 511
Celluloses, 226
Cement, of teeth, 557
Cephalin, 248–249, 605
Cephalopoda, flesh of, 572, 604
Cerebrin, 607, 609–610
in pus-corpuscles, 364, 365
Cerebron, 605, 609, 611–612
cleavage products of, 611
preparation of, 611
properties of, 611
Cerebrosides, 605, 607, 609
Cerebrospinal fluid, 360–361
Cerolein, 239
Cerumen of skin, 845
Cetin, 238, 239
Cetyl alcohol, 239, 627, 845
Chalasa, 634
Charcoal bone, ability to absorb trypsin, 703
Charcot's crystals, 871
Charcot-Leyden crystals, 621
Chemical processes, plants and animals, 37
"Chemical tonus," 591
Chief cells. See Adelomorphic cells.
Chitin, 122, 839, 839–840
Chitosamine, 219, 626
Chitosan, 168, 840
Chitose, 221
Chloral hydrate, fate of, in organism, 777
secretin, 415, 500
Chloramine, 40
Chlorbenzene, behavior in animal body, 786
Chlorides. See Mineral substances.
of urine, 758–761
in urine, quantitative estimation of, 759
quantity of, 759
Chlorine, in teeth, 558
in blood, 333
Chlorochrome, 384
Chlorocruorin, 299
Chloroform, behavior in animal body, 760, 775
influence upon elimination of chlorine, 760
influence upon muscles, 591
influence upon protein, 96
Chlorometer, 762
Chlorophan, 617
Chlorophyl, 38, 844
Chlorosis, 340
Chlortoluene, behavior in animal body, 783
Cholagogues, 415
Cholecyanin, 430
Choleprasein, 434
Cholepyrrhin, 427
Cholera bacilli, behavior toward gastric juice, 485
blood, 334
perspiration, 848
Cholestanol, α and β, 446
Cholestenon, 443
Cholesteriline, 445
Cholesterin, 10, 265, 613

Cholesterin, amounts in blood of different animals, 328
constitution of, 445
derivatives of, 446
ester, 264, 360, 444, 445, 613. 844
importance of, 449
in ascitic fluid, 359
in chyle, 347
in lymphatic glands, 366
in lymph, 348
in pus corpuscles, 364
in pus serum, 363, 365
in spleen, 370
occurrence of, 446
of sebum of skin, 845
preparation of, 450
properties of, 446
tests for, 447–448
Cholesterone, 445
Choline, 240, 246–248, 607
in cerebrospinal fluid, 360
in thyroid, 373
occurrence of, 247
preparation of, 247
properties of, 247
Cholohæmatin, 435
Chondrigen, 546
Chondrin, 121
Chondrin-balls, 549
Chondroalbuminoid, elementary composition of, 551
Chondroproteins, 168 ,172–174
Chondromucoid, 172, 546–547
elementary composition of, 551
preparation of, 548
Chondroitin, 547
Chondrosin, 171, 547
Chorda saliva, 448
Choroid coat, 619
Chromaffine tissue, 378
Chromhidrosis, 849
Chromogens. See Urinary pigments.
Chromoproteins, 92, 93, 167
Chyle, quantitative composition of, 346–347
Chyluria, 827
Chylous ascites, 358
Chyme, 478
Chymosin, 474
Ciamician and Magnanini's reaction for indol, 158
Circulating proteins, 908–910
Cleavage, hydrolytic, 16
processes, 15
Clupeine, 110
Coagulation, intravascular, 324
method for quantitative protein in urine, 793
Coagulins of blood, 320, 321
Coagulose, 59
Coaguloses, 135
Coapeptides, 136
Coaproteoses, 136
Cobra-poison, 310, 320
Cochineal, 844
Cocosite, 581
Codfish, eggs, 630
sperms, 107
Co-enzymes, 60
Coffee, action on metabolism, 912
Coilin, 92
Collagen, 92, 549, 551
analysis of, 118
in lymphatic glands, 366
preparation of, 118, 121
properties of, 119
Colloid, 171, 624, 625
cysts, 624
effect of charge upon, 23
effect of various ions upon, 22
envelope, 45
from uterine fibroma, 627
substances, non-permeability of, 9
suspension, electrolytic precipitation, 21
" Colloidal nitrogen," 795
substances, preparation of. 14
Colloids, 49
adsorption, 27–30
boiling-point, elevation of, 17
character of, 14
classification of, 15
diffusion of, 18
disperse phase, 27
dispersion means, 27
effects of the different ions, 25
electrical transportation of suspended particles, 20
electrolyte precipitation of, 24
emulsion, 15
examples of, 14
filterability of, 17
freezing-point, depression of, 17
hydrophile, 15
in relation to surface tension and adsorption, 24
irreversible, 21
migration of to poles, 21
molecular movement of, 20
optical properties of, 19
precipitation of, 21
precipitation phenomena, theories of, 25
protective, 23
relationship to crystalloids, 14
relative size of, 18
reversible, 21
suspension, 15
suspension, precipitation of, 70
Tyndal's phenomenon, 19
ultra microscope, use of in, 19
Colon, effect of extirpation of, 549
Coloring matter. See Pigments.
Colostrum, 658
composition of, 658

Colostrum of woman's milk, 665
Combustion, physiological heat of, 879
Complements, 69
Conalbumin, 633
Conchiolin, 122, 123
Concrements, intestinal, 523, 524. See also Calculi.
prostatic, 623
Concretions of lungs, 871
Conglutin, 878, 879
Conjugated glucuronic acids, fate of in organism, 777
sulphuric acids, fate of in organism, 777
Connective tissue, analysis of, 545
components of, 544
fibrils of, 545
Copper, occurrence in blood, 268, 333
in bile, 416, 433, 440
in liver, 387
in pigments, 299
Cornea, 550
Corneal mucoid, elementary composition of, 551
tissue, 619
Cornein, 122, 123–124
Cornicrystallin, 123
Corpora lutea, 623
Corpse-wax, 560
Corpus callosum, 613, 614
Corpuscles, blood. See Blood corpuscles.
colloid, 624
colostrum, 645
Gluge's, 624
Corpuscula amylacea, 612
Cover cells, 456, 476, 478
Crab extract, 576
Crangitine, 578
Crangonine, 578
Creatine, 267
detection of, 576
formation of, in active muscles, 594
in organism, 787
in ascitic fluids, 359
in urine, 692
mother substance of, 574
origin of, 574
preparation of, 573, 576
production from arginine, 575
properties, 575–576
relation of to creatinine, 692–693
relation to catabolism of protein, 574
Creatinine, 572, 573–576
elimination, effect of disease upon, 694
effect of muscular activity upon, 694
effect of starvation upon, 694
Folin's colorimetric method for, 697
Creatinine, formation of, in active muscles, 594
mother substances of, 693
preparation of, 697
properties of, 695, 696
quantitative estimation of, 697
quantity of, in urine, 692
relation of, to creatine, 692–693
-zinc chloride, 695
Crenilabrine, 110
Crenilabrus pavo, 844
Croners-Conheim's test for lactic acid in gastric juice, 488
Crude fibre, digestion of, 549
silk, 121, 122
Cruor, 252
Cruorin, purple, 282
Crusocreatinine, 578
Crustaceorubin, 844
Crusta inflammatoria, 306
phlogistica, 306
Cryptopyrrol, 430
Crystalbumin, 618
Crystalfibrin, 618
Crystallin, alpha and beta, properties of, 618
Crystalline lens, 617–619
Crystalloids, 13
Crystals, Charcot-Leyden, 621
Cuorin, 249
Curare, 348, 398, 588, 920
Cyanhæmoglobin, 284
Cyanhydrins, formation of, 200
Cyanmethæmoglobin, 284
Cyanocrystallin, 637, 844
Cyanurin, 741
Cyclopterine, 110
Cymene, fate of, in organism, 779
Cyprinine, 110
hexone bases in, 165
Cysteine, 80, 85, 148, 150, 619
Cystic fluid, protein bodies in, 626
Cystine, 80, 85, 106, 107, 113, 114, 115, 116, 125, 148–150
in urine, 827-828
preparation of, 828
protein-, 148
stone-, 148
Cystinuria, 827, 828
Cysts, characteristic constituents of, 624
colloid, 624
dermoid, 627
intraligamentary, 627
myxoid, 624
papillary, 627
parovarial, 627
proliferous, 624
serous, 624
tubo-ovarial, 627
Cytidine, 180, 185
Cytin, 368
Cytoglobin, 307, 315, 366
Cytosine, 178, 181, 185, 193–194

Cytosine, detection of, 194
Cytotoxin, 69
Cytozym, 319

Deamidation, 411, 536, 582, 702, 774
Dehydrochloride hæmin, 293
Dehydrocholon, 418, 423
Deniges's test for tyrosine, 154
Dentin, 553
Dermocerin, 844
Dermolein, 844
Desamidoprotein, 78
Descemet's membrane, 171, 551, 618
Desoxyhæmatoporphyrin, 291
Deutercaseoses, 129
Deuteroelastose, 117
Deuteromyosinose, 130
Deuteroproteose, 129
Deuterospongenose, 122
Deuterovitellose, 130
Development, work of, 642
Dextrin, 229
  hydrolytic cleavage products of, 229
Dextrins, 226
Dextrose, 212. See Glucose.
Diabetes, duodenal, 406
  mellitus, 403
    acetone bodies in, 818
  pancreas, artificial, 405
    relation to adrenals and thyroids, 406
  phlorhizin, 400
  sugar eliminated in, origin of, 409–411
Diabetic sugar, 212
Diaceton alcohol, 35
Diamine, 24, 131, 163, 757, 928
Dialysis, 14
Diarginylalanine, 111
Diarginylproline, 111
Diarginylserine, 111
Diarginylvaline, 111
Diastase. See Enzymes, 48, 64.
  action of, on starch paste, 226
Diazoacetic ether, 34
Diazobenzenesulphonic-acid test for dextrose, 216
Diazo-reaction, Ehrlich's, 755
    for histidine, 161
Dibenzoylornithine, 161
Diet, average daily adult, 923
  for people in different vocations, 924, 927
Diffusion, 1
  of colloids, 18
  streams, 5
Digestion, effect of extirpation of pancreas upon, 531–532
  gastric, effect of fats on, 481
  in the stomach, 478–489
    time of, 481
  movement of food in stomach during, 479–480
Digestion, movements of stomach during, 479
  peptic, 132
Digestion-work, 930–931
Diglucosamine, 220
Diglycyl-glycine, 85
Dihydrocholesterin, 443, 446
Di-isobutyldiacylpiperazine, 142
Di-iodotyrosin, 123
Di-leucyl-cystine, 85
Di-leucyl-glycyl-glycine, 85
Dimethylaminobenzaldehyde, fate of, in organism, 786
Dimethylfulvene, 7
Dimethylguanidine in urine, 758
Dimethylindol, 729
Dimethylketone, 822
Dimethyltoluidine, fate of, in organism, 786
Dimethylxanthine (1, 7), 713
Dioxyacetone, 205
Dioxybenzene, 778
o-Dioxybenzene, 727
p-Dioxybenzene, 728
Dioxymethylene creatinine, 574
Dioxynaphthaline, 778
Dioxypurine, 188
Dioxypyrimidine, 220
Dipalmitylolein, 233
Dipentosamine, 220
Dipeptides, 85–89
Diphosphatides, 239
Disaccharides, 223–226
Disperse phase, 47
Dispersion means, 47
Dissociation, degree of, 5
Di-stearyl lecithin, 148, 241, 242
Di-stearylolein, 233
Di-stearyl-palmatin, 233
Di-tetraoxybutylpyrazine, 220
Dithiopiperidine, 88
Donné's pus test, 798
Dotterplättchen, 93, 628, 638
Dulcite, 198
Dye stuffs, behavior of living cells toward, 10
Dyslysins, 427
Dysoxidizable substances, 4
Dyspeptone, 472
Dysproteoses, 129

Ear, fluids of the inner, 619
Earthy phosphates. See Phosphates, earthy.
Echinochrom, 299
Echinococcus cysts, fluid of, 362
Eck's fistula operation, 398, 537, 542, 680
Edestan, 108, 468
Edestin, 84
  cleavage products of, 107
  hexone bases in, 165
Eel-meat, 601
  serum, 250, 327
Egg shell, 636–641

Egg, white of the, 632–636
yolk of hen's, 628
Eggs, chemical energy in, 639
development of, 637
fertilization of, 639–640
incubation, 637–638
change in solid content during, 638
exchange of gases, 637–638
Loeb's experiments on fertilization, 639
Ehrlich's diazo reaction, 753, 847
glucosamine test, 221
side chain theory, 67, 71
test for bilirubin, 429
urine test, 826
Eicosyl alcohol, 844
Elaidin, 236
Elastic substance, action of trypsin on, 508
tissue, analysis of, 545
Elastin, 92
analysis of, 116
effect of gastric juice on, 473
hexone bases in, 165
in lymphatic glands, 366
peptone, 117
preparation of, 117
properties of, 117
Elastoses, 130
Electrolytes, amphoteric, 90, 93
Elephant, bones of, 553
Emulsin, 48, 58, 59, 62, 64
Emulsoids, properties of, 15
Enamel of teeth, 557, 891
Encephalin, 607, 609, 610
Endocrinic glands, 374, 375
Endoenzymes, 52
Endolymph, 619
Energy content of various food stuffs, 886
development, calculation of, 889
exchange, 891
calculation of, 889
metabolism calculation of, 886, 887
Enterokinase, 496
Enzymes, 37–70
action on glucosides, 62
action, specificity of, 61
action, retardation of, 62
activators, 52
adenase, 703
alcoholases, 875
aldehydases, 875
amylopsin, 501
anti, 63–64, 266
antipepsin, 467
arginase, 682
butyrinases, 266
catalases, 266, 872
classification of, 47
co-, 52
deamidizing, 703
Enzymes, deviation, 64
diastases, 266
effect of bile upon, 511–512
endo-, 52
esterases, 266
extra cellular, 52
fat splitting, 501–503
fermentation, 653
formation of, 52
gastric lipase, 476–478
general properties, 48
glutinase, 505
glycolytic, 332
glyoxylase, 584
guanase, 703
heat production of, 54
histozyn, 723
hydrogenases, 876
in amniotic fluid, 642
in ascitic fluids, 359
in blood, 52
in blood serum, 266
in bile, 435
in brain, 606
in fatty tissue, 558
in gastric juice, 466
in intestinal juice, 491–492
in leucocytes, 307
in liver, 386
in lungs, 870
in lymph, 346
in mammary glands, 643
in milk, 653
in muscle, 572
in pancreatic gland, 495, 500
in pancreatic juice, 496
in placenta, 641
in prostate, 621
in pus cells, 365
in pyloric secretion, 478
in saliva, 455
in spleen, 370, 371
in thymus, 369
in thyroid gland, 373
intracellular, 52
in urine, 757
in yolk, 628
lipases, 260
maltase, 266, 458
modes of action of, 54
myosin ferment, 570
nuclease, 504
nucleases, 703
oxidases, 266,
oxidones, 875
oxygenase, 872
pancreas press juice, action of, 62
pancreatic rennin, 509
pepsin, 466–476
peroxidases, 872
phenol-oxidases, 875
phytase, 579
polypeptide-splitting, 266

Enzymes, proteolytic, 82, 266, 703
pseudopepsin, 466
ptyalin, 456–460
purine-oxidases, 875
quantitative determination of, 58
reactivation, 52, 64
reductases, 876, 877
rennin, 266, 474
retardation by charcoal, 62
retarding substances, 65
reversibility of enzyme action, 58
salivary diastase, 456
Schütz's rule for, 58
secretion of, 52
steapsin, 501–503
syntheses, 38, 39
synthesis of hippuric acid, 39
trypsin, 503–509
tyrosinases, 875
urease, 829
uricase, 706
uricolase, 706
urocolytic, 706
xanthin oxidase, 703
Enzymotic processes, 40
fermentation processes, 41
hydrolytic cleavages, 40, 58
synthetic processes, 58
reactions, 55
laws of, 55, 56, 57
Epidermis, 112, 834, 835, 845
Epidermoidal structures, 837–838
Epiguanine, 187
Epiguanine in urine, 712, 714
Epinephrin, 378
Episarkine, 187
in urine, 712, 714
Epitoxiod, 71
Equilibrium constant, 33
nitrogenous, 906
Erepsin. See Enzymes, 48, 593–494
action of, 493
Ereptases, 503
Erythrite, relation to glycogen formation, 393
Erythrocytes, 275
Erythrodextrin, 229
Erythropsin. See Visual purple, 615
Esbach's quantitative method for protein in urine, 794
Ethal, 239
Ether, action on blood, 273
Ethyl alcohol, action on metabolism, 912
behavior in animal body, 775, 912
benzene, behavior in animal body, 778
butyrate, enzymotic synthesis of, 60
mercaptan, behavior in animal body, 776
secretin, 823
sulphide, from protein, 79
Ethyl sulphide, behavior in animal body, 776
sulphuric acid, behavior in animal body, 776
Ethylene glycol, glycogen formation, 394
Euglobulin, 259
Euxanthon, fate of, in organism, 785
Excelsin, 108, 160
Exchange of force, 871, 877, 879
Excrements, 521, 549, 873, 874
in biliary fistulas, 519
Excreta, regular and constant, 879
analysis of, 880
nitrogenous constituents of, 880
Excretin, 523
Exostosis, 556
Expectorations of lungs, 870–871
Extirpation of large intestine, effect of, 548
Extra cellular enzymes, 52
Extractive bodies of brain, 606
of kidneys, 673
of mammary glands, 643
of milk plasma, 647
non-nitrogenous, 579
of pancreatic gland, 495
substances of liver, 386
of muscles, 572–588
Extractives nitrogenous, of muscle, 572
of bone marrow, 554
of bodies of cystic fluids, 627
of testes, 620
of yolk, 628
Exudates, gases of, 857
Eye, fluids of, 615–619
lens of, insoluble protein of, 618
soluble protein of, 618
pigments of, 615–617
tissues of, 615–619

Fat-cells, membrane of, 558
action of trypsin on, 508
-globule, 646
Fats, 38, 232–249
absorption of, 535–540
effect of bile upon, 536–540
acetone formers, 820, 822
amounts in blood of different animals, 328
catabolism in starvation, 894
chemical methods for investigating, 238
deposition of, 920
destruction of, during work, 596
detection of, 237
effect of extirpation of pancreas on absorption of, 539, 540
effect of gastric juice on, 473
effect on glycogen content of liver, 394
emulsion of, 511
fate of, in organism, 885

Fats, formation from carbohydrates, 563
from glycogen in liver, 397
from protein, 560–562
of, in organism, 559
hydrolysis of, 234
human, 559
in blood, 334
serum, 265
in chyle, 346, 347
in kidney, 673
in liver, 384
in lymph, 348
in lymphatic glands, 366
in muscle, 586
in pus corpuscles, 364
in pus-serum, 363
in spleen, 370
in synovial fluid, 362
in thymus, 368
in urine, 827
in woman's milk, 660
in yolk, 630
manner of absorption of, 535–536
metabolism of, in starvation, 894
with an exclusive protein diet, 907–908
muscular energy, source of, 598
of different animals, 559
pancreatic splitting of, 502
properties of, 233
saponification of, 234
storing up of, 563
syntheses of, 60
Fatty degeneration, 385, 560
series, fate of, in organism, 773–774
tissue, 558–564
analysis of, 558
constituents of, 558
Feathers, mineral substances of, 838
pigments of, 843–844
Feces, appearance of, 521, 522
constituents of, 521, 879
pigments in, 522
reaction of, 522
Feeding experiments to show value of different foodstuffs, 904–906
Fermentation, 8, 9, 203, 207
lactic acid, in stomach, 485
processes, 41
test in urine, 803, 804, 809, 810, 812
for sugar, 803
Ferments, 41
Ferratin, of liver, 383
Ferrine, 384
Fertilization membrane, bringing about, 640
Fever elimination of ammonia. 768
Fibrin, 106, 251, 254–261
action of, 257
cleavage products of, 106
coagulation, 256, 257
elementary composition of, 263
Fibrin, ferment, 256
globulin, 258, 259
Henle's, 620
in blood coagulation, 317
in blood during pregnancy, 337
in venous blood, 336
manner of formation, 322
peptic digestion of, 472
plastic substance, 258
preparation of, 254–255
production, manner of, 315
properties of, 255
quantitative estimation of, 255
Fibrinogen, 91, 252–261
amounts in blood, in poisoning, 253
detection of, 254
elementary composition, 263
formation, seat of, 252
in coagulation of blood, 317
in venous blood, 336
occurrence of, 252
preparation of, 254
properties of, 253
purification of, 254
quantitative estimation, 254
relation to fibrin, 257, 258
transformation into fibrin, 256
Fibrinolysis, 255, 322
Fibroin, 92, 122, 124
analysis of, 125
properties of, 124
Filterability of colloids, 17
preparation of the filter, 18
Fischer-Weidel's reaction, 189
Fleischl's hæmometer, 299
Florence's sperm reaction, 621
Fluorine content in teeth, 558
content in organs and tissues, 553
Folin and Dennis' test for tyrosine, 154
Folin's method for urea, 689
Folin-Schaffer's quantitative method for uric acid, 711–712
Food, amount of, for an average daily diet, 933, 934
chemical energy introduced with, 932
definition of, 878
influence on blood, 338
necessity under various conditions, 932–939
needs of, in work and rest, 937–939
requirements for men in various vocations, 933
stuff, determination of heat value, 891
energy content of, 886
organic, uses for in organism, 890
physiological availability of, 891
Foods, energy of, 885
essential to life, 878

Foods, importance of various, 878
Formaldehyde, 38
formation of, from carbonic acid, 2
relation to glycogen formation, 397
transformation into sugar, 1
Formol titrable nitrogen in urine, 755, 756
titration, Sörensen's, for amino acids, 166
Freezing-point depression, for mammalians, 12
in the frog, 12
in the invertebrates, 12
in the lower fishes, 13
in the higher fishes, 13
in the eel, 13
in urine, 13
of colloids, 17
molecular lowering of, 4
Fructosazine, 221
Fructose, 217–218
in blood serum, 265
in urine, 814–815
structural formulæ for, 197, 199
tests for, 217–218
Fruit-sugar, 217. See Levulose.
Fuld and Levison's method for testing pepsin, 470
Fürbringer's test for proteid, 790
Furfurol, 208–209
fate of, in organism, 784
Fuscin, 617

Gadushistone, 108
Galactosamine, 220
Galactose, 654, 816
structural formula for, 199
tests for, 217
Galactoside, 202
Gall bladder, secretion of, 416, 437
Gall-stones, 444
Gallois' inosite test, 578
Ganassini's reaction for uric acid, 707
Gas exchange, a measure of metabolism, 927–928
between blood and pulmonary air, 858–870
between blood and tissues, 858–870
in muscle activity, 592
in starvation, 895
methods for the quantitative determination of, 868–870
through skin, 849
rise of, 931
tension in blood, 859–868
methods of determining, 864–866
Gases, in bile, 857
in birds' eggs, 636
in blood, 857, 850–856
in exudates, 857
in blood serum, 269
in gastric digestion, 485–486
in lymph, 346, 856–858,
in milk, 657, 658
in muscles, 588
in saliva, 857
in stomach, 485–486
in transudates, 355
in urine, 770, 857
in woman's milk, 664
produced in putrefaction, 515–516
Gastric contents, indicators for determining acids in, 487
nature of acids in, 487
quantitative determination of lactic acid in, 488
test for lactic acid in, 488
digestion, absorption of cleavage products in stomach, 484
degree of, 483, 484
effect of fats on, 481
gases in, 485, 486
time of passage through the stomach of different foods, 482, 483
juice, 461–466
action of foreign substances on secretion of, 462
action of saliva on secretion of, 463
composition of, 465
constituents in, 466
degree of acidity in, 487
obtainment, free of saliva 461–462
origin of hydrochloric acid in, 477
secretion of, 461, 462–463
in man, 464
lipase, 476–478
Geissler's albumin-test papers, 790
Gel, 15
Gels, 30–32
Gelatin, 31, 78, 80, 118, 119–121, 152
analysis of, 118
as a foodstuff, 912
forming substances of bones, peptic digestion of, 472
forming substances of cartilage, peptic digestion of, 472
forming substance of connecting tissue, action of trypsin on, 508
forming substances of connective tissue, peptic digestion of, 472
from peptic digestion, 473
hexone bases in, 165
in egg development, 639

Gelatin, in protein catabolism, 911
oxidation of, 83
pancreatic digestion of, 508
peptic digestion of, 473
peptones, 120, 121
preparation of, 118
properties of, 119
protein-sparer, 911, 912
Gelatose, proto-, 120
deutero-, 120
Gelatoses, 120, 473
Generation, organs of, 620–642
Generative organs, female, 623–642
secretions, male, 620–623
Gerhardt's test for acetoacetic acid, 824
Glands, albuminous, 541
Brunner's, secretion of, 489
fundus, 460, 461
Lieberkuhn's, secretion of, 490
mammary, 643
constituents of, 643
mixed, 451
mucous, 451, 460
membrane, in the intestine, 489–493
membrane of stomach, 460
pancreatic, 494–495
pyloric, 460, 461
salivary, 451–460
analysis of, 451
Gliadin, cleavage products of, 107
Globan, 104, 276
Globulins, 78, 91, 92, 93, 106
detection of, in urine, 791
properties of, 104
quantitative estimation of, in urine, 791, 793
Globuloses, 130
Glucocyanhydrin, 200
Glucomaines in urine, 757, 758
Glucoproteins, 167, 168–174
Gluconose, 206
Glucopeptose, 200
Glucoproteins, phosphorized, 105
Glucoproteose, 133
Glucosamine, 84, 219
from blood globulin, 260
from seralbumin, 262
preparation of, 220
tests for, 219
d-glucosamine, 201
Glucosan, 213
Glucose, 59, 212–217
in blood, 330
in urine, 749, 802–814
from blood globulin, 260
osimine formation, 201
preparation of, 216
properties of, 213
structural formula for, 197, 198, 199
tests for, 213–216
Glucosides, 202
Glucosides, action of enzymes on, 62
Glucoside-splitting enzymes, 16, 202, 491
Glucosoxime, 200
Glucuronates, conjugated, properties of, 751, 752
conjugated in urine, 750–752
Glucurone, 222
Gluge's corpuscles, 623
Gluteins, 119
Glutelins, 92
Gluten casein, hexone bases in, 165
proteins, hexone bases in, 165
Glutin, 92
Glutinase, 505
Glutokyrin, 138
Glycerides, tri-, 232
Glycerine, 265
relation to glycogen formation, 394, 397, 412
Glycine. See Glycocoll, 139
Glycocoll, 85, 106, 107, 109, 113, 115, 119, 124, 125, 139
amounts in proteins, 106, 107, 113, 115, 125
conjugation with, 782–785
formation of, in organism, 721
importance of, in uric acid formation, 720, 721
Glycocyamine, 693
Glycogen, 229, 390–414, 581–582, 637
amount in liver, 390, 391
amount in muscles, 390, 391
consumption of, in muscles, 592
content increased by, 393, 394, 396, 397
fat formation from, 397
formation, a cell function, 398
formation from sugar, 395
in lymph, 346
in lymphatic glands, 366
in placenta, 641
origin of, 393
origin in muscles, 397
preparation of, 392
properties of, 391, 392
pseudoglycogen-formers, 395, 396
quantitative estimation of, 393
synthesis of, 58
synthesis of, in liver, 381
true glycogen formers, 395, 396
transformation into sugar, 398, 399
Glycolaldehyde, 38
Glycolysis, 265, 332, 407–409, 570
Glycolytic enzyme, 265
Glycoproteins, 92, 167, 168–174
in blood, 264
Glycosuria adrenalin, 402, 403
relation of pancreas and adrenals, 406
alimentary, 401
diabetic, relation of pancreas to, 405

Glycosuria piqure, 402
salt, 401
sugar-puncture, 402
Glycylalanine, 86
Glycylasparaginyl leucine, 86
Glycylglycin, 86
Glycyl leucine, 86
proline anhydride, 86
tyrosine, 86
valanine anhydride, 86
Glyoxal-methyl, 202
Glyoxyldiureide. See Allantoin, 717
Gmelin's test, for bile pigments, 429
in urine, 800
Goitre, 376
Gorgonin, 123
Graafian follicles, 623, 624
Grape sugar, 212. See Glucose.
Guaiac test for blood, 281, 795
Guanadine, 162
Guanase, 48, 188, 187, 382, 368, 702. See Enzymes.
Guanidobutylamine, 162
Guanine, 178, 181, 183, 185, 187, 188, 190, 191, 712
Capronica's test for, 191
epi-, 187
-hexoside, 180
Weidel's reaction for, 191
Guano, 188, 699
Guanosine, 180
Guanovulit, 637
Gulose, 211
Gums, 229, 230
plant, 226
vegetable, 230
Gunning's modified Lieben's test, 822
Gunzburg's test for HCl in gastric juice, 487
Gynesin in urine, 758

Hæmaphilia, 329
Hæmase, 50
Hæmatäerometer, 861
Hæmatin, 289–291
neutral, 215
reduced, 288
Hæmatinogen, 296
Hæmatinometer, 297
Hæmatocrit, 326
Hæmatocrystallin, 278
Hæmatogen, 629, 637
Hæmatoidin, 871
Hæmatoblasts, 308
Hæmatoporphyrin, 294–296, 844
relation to bilirubin, 295, 428, 440
relation to chlorophyl, 276, 295
relation to urobilin, 295, 440, 742
in urine, 740, 797–798
Hæmatoscope, 303
Hæmaturia, 795, 796
Hæmerythrin, 299
Hæmin, 292–294, 297, 800
crystals, 292, 293, 800
Hæmochrom, 275, 279
Hæmochromogen, 276, 288, 289
Hæmocyanin, 92
Hæmoglobin, 92, 108
amounts in blood, 328
carbon dioxide binder, 853
exudation of, 6
in blood during pregnancy, 337
in CO_2-O_2 exchange in blood, 853
in venous blood, 326
transformation into bile pigments, 442
Hæmoglobinuria, 796
Hæmoglutination, 275
Hæmolysins, 69
Hæmolysis, 273
Hæmometer, 303
Hæmopyrrol, 276, 291, 295, 428, 843
Hæmorrhodin, 287
Hæmoverdin, 287
Hair, 837
-balls, 524
human, sulphur content of, 838
lanugo, 642
Hammarsten's test for bile pigments, 430, 800
Haptogen-membrane, 646
Haptophore, 67
Hanriot and Richet's method for determining respiratory exchange, 869
Häser's coefficient, 771
Heat regulation, chemical, 929
physical, 929
Hedenius' bilirubin test, 433
Helicoproteid, 174
Heller's blood test, 797
scheme for investigating calculi, 837
Heller-Teichmann's test, 797
in urine, 788
for proteid, 98
Hemicelluloses, 231
Hemicollin, 120
Hemielastin, 117
in blood, 264
Hemipeptone, 130
Hemolysins, 70
Hemp seed calculi, 835
Henle's fibrin, 620
Hénocque's hæmatoscope, 299
Henriques and Gammeltoft's method for urea, 690
Heparphosphatide, 386
Hepatopancreas, 494
Heptapeptides, 85, 87
Heptose in urine, 817
Heptoses, 197
Heterocaseoses, 129

Heterocyclic compounds, fate of, in organism, 778–787
Heterolysis, 18
Heterosponginose, 122
Heterosyntonose hexone bases in, 165
Heteroxanthine, 187
in urine, 712, 713
Hexapeptides, 85
Hexobioses, 224
Hexone bases, 161
in various proteins, 165
Hexoses, 197, 211–218
syntheses of, 212
Hippokoprosterin, 449
Hippomelanin, 841
Hirudin, 251
Histidine, 85, 106, 107, 109, 110, 111, 115, 117, 119, 125, 159-161
diazo reaction for, 161
in various proteins, 165
Weidel's reaction for, 160
Histidyl-histidine, 85
Histone in urine, 795
Histone-peptone, 109
Histones, 78, 91, 92, 93
Gadus-, 108
hexone bases in, 165
Lota-, 108
properties of, 108–109
Histozym, 48
Hoffmann's test for tyrosine, 153
Holozym, 319
Homocerebrin, 607, 609, 610
Homocyclic compounds, fate of, in organism, 778–787
Hopkin's quantitative method for uric acid, 711
Hoppe-Seyler's reaction for xanthine, 190
CO blood test, 286
colorimetric method, 297
test for bile acid, 179
Hordein, 107, 156
Hormone, 375
Hormones, 407
Horn structures, 837
mineral content of, 838
sulphur content of, 838
See also Keratin.
Hüfner's bile, 419
Humor, aqueous, 352, 359, 617
Huppert-Messinger method of estimating acetone, 825
Huppert-Schültz's method of testing pepsin, 468
Huppert's test for bile pigments, 429, 430, 799
Hyalin, 840
Hyalines, 171
Hyaline substance, 364
Hyalogens, 171
Hyalomucoid, 617
Hydantoins, 786
Hydræmia, 340, 352
Hydramnion, 642
Hydrazine poisoning, 718
Hydrazones, 202–203
Hydrobilirubin, 743
Hydrocele and spermatocele fluids, 359, 360
Hydrochinon in urine, 727
Hydrogel, 14
Hydrogen, colorimetric method, 75
determination of, 75
determination of, in fluids containing CO_2, 76
electromotive method, 75
ion content, 75
peroxide, 35
Hydrogenases, 876
Hydrolytic cleavage processes, 40
Hydroquinone, 727
Hydrosol, 14
Hydroxylamine poisoning, 718
Hyperacidity, 486
Hyperglycæmia, 401, 402
Hyperthyreoidismus, 378
Hypertonic solution, 6
Hypnotics and glycogen formers, 394
Hypophysis, 380
Hypotonic solution; 6
Hypoxanthine, 178, 187, 188, 191, 192, 572, 712
detection of, 191

Ichthidin, 630, 636
Ichthin, 636
Ichthulin, 92, 174, 630, 636
Ichthylepidin, 122
Icterne, 413, 428, 431, 440, 441
urine, 799
Ignotine, 576
Ileum, extirpation of, 549
Imbibition, 30
Imidazol derivatives in urine, 757, 826
structural formula of, 186
Immune bodies, 66, 69
Immunity, 63–70
active, 70
passive, 70
theory of, Arrhenius', 67, 68
theory of Erlich, 67, 68
Immunization, 66
Indican, 517, 728
elimination of, 728
effect of putrefaction on, 729
excretion of, 729, 730
quantitative method for, 731
tests for, 730, 731
Indigo-blue of urine, 741
Indigo-red formation, source of, 731
Indigotin in urine, 728, 731
Indirubin in urine, 731
Indol, 46, 82, 117, 157–159, 267, 515, 522, 843
fate of, in organism, 786
formation of, in organism, 728, 729

Indol, methyl-, 157
tests for, 158
Indoxyl, 724
Infraproteins, 93
Inosine, 180, 577
Inosite, 579–581
in adrenal bodies, 377
preparation of, 580
properties of, 580
Inositogen, 577
"Integral factor," in uric acid elimination, 705
Internal friction, 18
effect of acids and alkalies upon, 19
effect of NaCl upon, 19
of hydrophile colloids, 19
of suspension colloids, 18
Intestinal fermentation, 512, 513
juice, constituents of, 491, 492
properties of, 491
putrefaction, 512, 513
Intestine, chemical processes in, 510–524
decomposition by microbes in, 513
large, putrefaction in, 515
small, digestion and absorption of various foods in, 513–514
walls of, chemical processes in, 527–529
Intracellular enzymes, 52
Intraligamentary cysts, 627
Inulin, 228
Inversion of sugars, 224
Invert sugar, 224
Invertase. See Enzymes, 48
Invertin, 225. See Enzymes.
Iodine combinations, passage into milk, 667
passage into perspiration, 849
passage into saliva, 457
in blood, 268, 333, 337
in glands, 378, 379
in perspiration, 848
in proteins, 81, 125
Iodoform, fate in organism, 795
Iodospongin, 124
Iodothyrin, 373, 376
of thymus, 368
Iodothyrioglobulin, 373, 376
Ions, 5, 70–76
action of, on enzymes, 70
Iron, absorption, 338–339
and blood, 339, 639
elimination, 433, 440, 770
importance of, in metabolism, 903
in spleen, 371
in urine, 769
results in lack of, 895
Islands of Langerhans, 407
Iso-casein, 649
Isocholesterin, 449
Isodynamic law in metabolism, 890–892
Isoelectric point, 21, 26
Isolactose synthesis of, 58
Isoleucine, 85, 106, 107, 143–145
Isomaltose, 223, 224, 225–226
in urine, 749
synthesis of, 58
splitting of, 58
Isoserine, 146
Isosmotic solutions, 3
Isotonic solution, 5
Isotropous muscle substance, 564

Jacoby's quantitative method for pepsin, 470
Jaffe's creatinine reaction, 695
Jaffe-Obermayer's indican test, 729
Janthinin, 844
"Jaune indien," 222
Jecorin, 605
in liver, 385
in spleen, 370
Jejunum, effect of extirpation of. 549
Jerusalem's test for lactic acid in gastric juice, 488

Kaolin, 16
Karyogen, 623
Kephir, 518, 654, 659
effect on putrefaction, 518
-lactase, 57. 58
Kerasin, 607, 609, 610–611, 613
Keratin, A, B, C of horn and hair, properties of, 837
action of gastric juice on, 473
Keratins, 92, 112–116
composition of, 113
Ketone, behavior in animal body, 776, 777
Ketoplastic action, 819
Ketoses, 197
Kidneys, constituents of, 672–674
formation of hippuric acid in, 722
quantitative analysis of, 673
relation to formation of urea, 686
Kinase, in thrombin formation, 323
occurrence in intestine, 492
Kinases, 51
Kjeldahl's method for total nitrogen, 688
Klupeovin, 698
Knapp's method of estimating sugar, 812
reaction for dextrose, 215
Knop-Hüpner's method for urea, 696
Koilin, 115
Koprosterin, 448
Kossel's reaction for hypoxanthine, 192
Kossler and Penny's method for phenolsulphuric acids, 726
Kramm's creatinine reaction, 696
Kumyss, 654, 659

Kyrin, 137
caseino-, 137
gluto-, 137
proto-, 137
Kyrines, 167
carbamino reaction with, 167

Lactacidase, 207
Lactalbumin, 91, 652–654
cleavage products of, 106
composition of, 652
properties of, 653
Lactase, 48, 64
Lactimide, 156
Lactocrit, 657
Lactoglobulin, 652
Lactones, 198
Lactoprotein, 653
Lactose, 223, 224, 647, 654–655
in milk, conversion into lactic acid, 644
in urine, 815
preparation of, 655
properties of, 654
tests for, 655
theory of formation in mammary gland, 643
Lactosuria, 811
Laiose in urine, 815
Langerhans, islands of, 494
Lanocerin, 844
Lanolin, 446
Lanugo hair, 642
Large intestine, effect of extirpation of, 548
Latebra, 624
Lead in blood, 333
in the liver, 389
passage into milk, 670
Lecithin, 105, 586
amounts in blood of different animals, 328
from blood serum, 261
in chyle, 347
in lymph, 348
in pus corpuscles, 364, 365
in pus-serum, 363
quantitative determination of, 246
Lecithins, 10, 242–249
hydrolytic products of, 342
in metabolism, importance of, 902
preparation of, 246
properties of, 244
structure of, 242
Lecithoproteins, 92
Legal's reaction for indol, 158
Legumin, cleavage products of, 107
Lense, of oxen, analysis of, 619
Leo's sugar, 810
Lepidoporphyrin, 844
Lethal, 238, 239
Leucæmia, blood, 187, 341, 698
Leucæmia, spleen, 375
urine, 710, 764
Leucine, 85, 106, 107, 109, 111, 113, 115, 123, 124, 125, 141–143, 267
conversion into isoamyl alcohol, 206
in brain, 607
in lymphatic glands, 366
in spleen, 370
in thyroid gland, 373
in urine, 755, 827
Leucinimide, 143
Leucocytes, 305
constituents affecting coagulation, 315
in venous blood, 336
osmotic phenomena with, 8
See also white blood corpuscles
Leucomaines, 25
Leuconuclein, 367
in blood, 316
Leucopolïn, 609
Leucyl peptides, 85–88
Levulins, 228
Levulose, 217
in urine, 814–815
Lichenin, 228
Lieberkühns solid alkali albuminate, 126
Lieben's iodoform test for acetone, 822
Liebermann-Bürchart's, for cholesterin, 447
Liebermann's reaction for protein, 100
Liebig's method for urea, 688
Lienases, 371
Lifschütz's reaction for cholesterin, 445
Ligamentum nuchæ, 115, 116, 546
Lignin, 231
Lime salts, manner of excretion, 769
Lipanin, absorption of, 545
Lipase, 47, 48
pancreatic, 57
Lipases, plants, 64. See also Enzymes.
Lipochromes, 268
Lipopeptides, 88
Lipoids, 241
as a special limiting layer, 10
in brain, 605
in relation to osmosis in cells, 10
sulphurized, 605
Lipuria, 827
Lithium, 22, 333
Liver, 381–450
acetone formation in, 819, 822
amino acids, synthesis of, 382
autolysis of, 387
assimilation in, 381
bile pigments, formation of, 381
blood pigments, destruction of, 388
-cells, reaction of, 382
conjugation of glucuronic acid in, 382
constituents of, 382

Liver, creatin-creatine metabolism, 694, 695
deamidation in, 382
destroying of uric acid in, 707
ethereal sulphuric acids, formation of, 382
formation of amino acids in, 530
formation of bile in, 382
glycogen formation, 381, 390
glycogen content in, extirpation of the adrenals, 402
glycolysis in, theories for, 408
iron in, 387, 388
" organ plasma " of, 382
pigments of, 384
processes going on in, 381, 382
protein synthesis in, 529, 530
storehouse for protein, 389
synthetical formation of uric acid in, 705
urea formation, 382
of, from ammonia, 767
uric acid formation in, 702, 703
Livetin, 629
Lotahistone, 108
Lungs, concretions of, 871
constituents of, 870
expectorations of, 870, 871
gas, exchange in, 867
Lutein, 631
Luteins, in blood serum, 268
Lymph, 345–380
amount secreted, 349
composition of, 345–348
coagulation of, 346
formation of, 350–352
gases of, 856–858
origin of, 345
osmotic pressure in, 348
secretion of, circumstances influencing, 349, 350
Lymphagogues, 350
Lymphatic glands, 366
quantitative composition of, 366
Lymphocytes, 300
Lymphoid cells, 305
Lysatine, 160
Lysatinine, 160
Lysine, 85, 106, 107, 109, 111, 113, 115, 117, 119, 123, 124, 125, 163, 164, 267
in various proteins, 165
peptone, 138
Lysines, 47
Lysylglycylpeptide, 89
Lysyl lysine, 85

Magnesium in urine, 768, 769
triphosphate in urinary sediment, 831
Maintenance value, 897
Maltase, 48, 458. See also Enzymes.
Malt dextrin, 229
Malt diastase, 225
action of, upon starch, 226
Maltose, 223, 224, 225
in urine, 815
synthesis of, 58
Malt sugar, 225
Mammary gland, theory of formation of lactose in, 643
Mandelic acid ester, racemic, 62
nitrile glucoside, 59
Manganese, 268, 333, 442
Mannite, 198
Mannose, 198, 216
Manonose, 206
Marcitine, 47
Margarin, 235
Maschke's reaction for creatinine, 695
Mass action, 32, 74
Mastic, 22
Meconium, 523
Medicinal coloring matters in urine, 801
Medulla, 607
Medullary fibers, composition of, 614
Melanin, 617, 842
in urine, 799
Melanins, of skin, 841
mother substances of, 843
Melanogen in urine, 799
Melanoidin nitrogen, 78
Melanoidins, 841
Melano-protein, 842
Melanotic cancers, 802, 842, 843
Membrane, semi-permeable, 26
Membranin, 171, 550
properties of, 617
Menthol, fate of, in organism, 785
Mercuric salts poisoning, 23
Mesitylene, fate of, in organism, 779
Mesoinosite, 582
Mesoporphyrin, 295
Mesoxalyl urea, 698
Metabolism, 878–939
affected by sleep, 928
as affected by external temperature, 929
as affected by high altitude, 930
as affected by ingestion of food, 930
as affected by light, 928
as affected by mental activity, 928
basal requirement, 897, 898
calculation of, 889
conditions affecting, 922–931
effect of age upon, 922–925
effect of alcohol on, 921, 922
effect of coffee and tea upon, 922
effect of rest and work upon, 926–928
effect of salts on, 921
effect of sex upon, 925, 926

Metabolism, effect of weight of body upon, 922–925
effect of water on, 920, 921
importance of lecithins in, 902
importance of phosphates in, 902
in active and inactive muscles, 591–598
inanition condition, 897–898
in starvation, 892–906
lack of mineral substances in food, 899, 900
water in food, 898
maintenance value, 897–898
measured by gas exchange, 927, 923
with absence of carbohydrates in food, 903, 904
with absence of fats in food, 903–904
with absence of proteins in food, 903
with a mixed diet, 913–920
of fat with an exclusive protein diet, 907, 908
with foods rich in protein, 906–913
with insufficient supply of chlorides, 900, 901
with lack of bases in foods, 901
with lack of earths in food, 901, 902
with lack of iron in foods, 903
with lack of phosphates in food, 901, 902
with various foods, 906–922
Metacasein, 651
Metalbumin, 624, 625
Metanitrobenzaldehyde, fate of, in the body, 784
Metaproteins, 93
Metazym, 319
Methæmoglobin of thyroid gland, 374. See also Blood pigments.
Methal, 239
Methose, 212
Methylation in organism, 787
Methylenitan, 212
Methylethylmaleic acid anhydride, 290
Methylglycocoll. See Sarcosin.
Methylglyoxal in relation to lactic acid fermentation, 584
Methylguanidine, 578, 698
in urine, 758
Methyl guanine (7), 714
Methylindol, 157 See Skatol.
Methylindolin, 729
Methylimidazol formation of, 201–202
Methyl pentoses, 199, 208
Methylphenylhydrazine, test for levulose, 218
Methyl pyridine, behavior in animal body, 778, 781
chloride in urine, 756, 758
Methylpyridylammonium hydroxide, 787
Methylthiophene, 784
Methyluracil, 194
Methyluramine, 574, 694
Methyl-urea, 691
in urine, 712, 713
Methylxanthine, 187
Mett's quantitative method for pepsin, 469
Micro-organisms in intestines, 511, 514, 517, 520
Microrespirometer, 874
Microtonometer, 861
Milk, 643–671
albumin, quantitative determination of, 656
ash, quantitative composition of, 666
casein, quantitative determination of, 656
coagulation, a two-faced process, 650
coagulation of, 645
cows, 644, 645
cows, quantitative composition of, 657
effect on putrefaction, 518
fat, quantitative determination of, 657
constituents of, 647
theory for origin of, 669
foodstuffs in, 666
gases of, 857
globules, 645–647
goat's, 659
human, 660–671
human, compared to cow's milk, 660, 661, 663
influence of food on composition of, 667, 668
mare's, 659
lactose quantitative determination, of, 657
mineral bodies, quantitative determination of, 656, 657, 658
of carnivora, 659
of various animals, composition of, 660
passage of foreign substances into, 670
plasma, 647
preparation, composition of, 659
proteins, quantitative determination of, 656
quantitative analysis of, 656, 657
secretion, chemistry of, 668–671
solids, quantitative determination of, 656
souring of, 645
sugar, 226. See Lactose.

Milk, sugar, theory for origin of, 670
quantitative determination of, 657
theory for origin of casein, 668
permanent emulsion, 646
uterine, 641
various fermentations of, 645
woman's, quantitative composition, 652, 653
quantity of mineral substances in, 654
Millon's reaction for proteins, 99
in hydrocele and spermatocele fluids, 359
Mineral bodies in milk, 656
Mineral substances, absorption of, 540
amounts in blood of different animals, 328
distribution of, 72
effect on metabolism, 921
importance to life of cells, 72
in bile, 436
in blood, 335
in blood serum, 268, 269
in bone structure, 552
in brain, 615
in cartilage, 550
in cells, 72
in cerebrospinal fluid, 361
in chyle, 347
in connective tissue, 546
in cystic fluid, 627
in egg shell, 636
in feathers, 838
in gastric juice, 466
in hair, 838
in intestinal juice, 491
in kidneys, 673
in liver, 387–389
in lungs, 870
in lymph, 346, 348
in muscles, 586–588, 599
in nails, 838
in pancreatic gland, 495
in pancreatic juice, 500
in pericardial fluid, 356
in pus corpuscles, 365
in pus-serum, 363
in retina, 615
in semen, 621
in smooth muscles, 602
in spermatozoa, 622
Mineral substances, in starvation, 895, 896
in synovial fluid, 362
in urine, 758–770
in white of egg, 635
in woman's milk, 654
in yolk, 632
lack of, in food, 899, 900
of spleen, 361
toxicity of, 73
Mingen in urine, 756, 758
Mohr's quantitative method for chlorides, 759
Molisch's test for sugar, 216
Monaminophosphatides, 241
Monomethylxanthine, 712
Monosaccharides, 197–218
cyanhydrin formation, 200
oxime formation, 200
transformation of, 201
Moore's test for sugar, 214, 224
Mörner-Sjöqvist and Folin's method for urea, 689
Morphine, elimination in urine, 749, 787
in milk, 670
Moss-starch, 228
Mucin, 619
action of trypsin on, 508
"dissolved," 794
effect of gastric juice on, 473
in urine, 757, 794
pseudo, 171
substances, 168–172
Mucilages, 226
vegetable, 230
Mucinoids, 171
Mucins, analysis of, 169
amino acids in, 170
cleavage products of, 170
true, 168, 169
Mucoid, osseo-, 172
Mucoids, 169, 171
effect of gastric juice on, 473
Mucous of urine, 674
Mulberry calculi, 833
Murexide test, 707
Muscarine, 246
Muscle-pigments, 571
Muscle-plasma, 566
preparation and properties of 566, 567
proteins of, 569 570
Muscle-serum, 566
smooth, 601–603
constituents of, 602
extractives in, 602
"snow," 566
"-stroma," 569
syntonin, 569
Muscles, 565–603
acid rigor of, 590
active, acid reaction of, 593

Muscles, active, characteristics of, 596
amount of fat in, 600
an isotropous substance of, 555, 556
chemical rigor of, 590
dead, proteins of, 567
elementary analysis of, 601
extractive bodies of, 572–588
"flesh quotient," 601
hæmoglobin, 571
heat rigor of, 590
imbibition rigor of, 590
isotropous substance of, 555, 556
metabolism in, 591–598
of different animals, ash analyses of, 599
of different animals, analyses of, 599
proteins of, 566–572
quantitative composition of, 598–601
reaction of, 565
rigor mortis of, 588–591
striated, 565–601
water rigor of, 590
Musculamine, 578
Muscular action, source of, 597
Musculin, 568, 570
Myelin, 608
"Myeline forms," 605
Myoalbumin, 569
Myocynine, 578
Myogen, 570, 571
Myogen-fibrin, 570, 571
soluble, 570
preparation of, 570, 571
Myoglobulin, 568, 571
Myohæmatin, 571
Myoproteid, 571
Myosin, 84, 567, 568, 570
as related to fibrinogen, 254
coagulation of, 570
ferment, 570
fibrin, 568, 570
in blood corpuscles, 306
preparation of, 568
Myosinogen, 569, 570, 571
Myosinoses, 130
Myrisin, 239
Mytilite, 581
Mytolin, 569
Myxœdema, 374
Myxoid cysts, 624

Nails, 837
mineral substances of, 838
Naphthalene, fate of, in organism, 779, 785
Napthoresorcin, reaction for conjugated glucuroric acid, 218, 223, 818
reaction for levulose, 218
Naphthylisocyanate compounds of amino-acids. See various Amino-acids.
Narcotics, relation to glycogen formation, 394
Neosine, 578
Neossin, 171
Neottin, 631
Neozym, 320
Nephrorosein in urine, 734
Nerve fibres, analysis of, 614
Nerves, 604–615
Neubauer's and Rolide's reaction for proteins, 100
Neuberg's test for levulose, 218
Neuberg-Rauchwerger's reaction for cholesterin, 444
Neuridine, 606, 612, 628
Neurine, 246
Neurochitin, 614, 615
Neuroglia, 601
Neuroglobulin, α and β, 604, 605
Neurokeratin, 605, 614, in various nerves, amounts in, 614
Nicotine, effect upon gases of stomach, 485
Ninhydrin reaction, 101
Nitrates in urine, 766
Nitriles, fate in organism, 775
Nitrobenzaldehydes, fate of, in organism, 783, 784
Nitrobenzene, fate of, in organism, 782
Nitrogen colloidal, 795
elimination in starvation, 893, 894
through various channels, 880, 881
gaseous elimination of, 881. See also Gases in various tissues and fluids.
Nitrogenous equilibrium, 918, 919, 934
Nitrotoluol, behavior in animal body, 786
Novaine, 577
in urine, 758
Nuclease, 504, 508
Nucleases, 48, 182. See also Enzymes.
Nucleinases, 182
Nuclein bases, 186
in lymphatic glands, 366
plates, 308
in pus-cells, 365
true, 176
Nucleins, 176, 177
action of trypsin on, 508
effect of gastric juice on, 473
pseudo-, 177
Nucleoalbumins, 91, 92, 93, 177
detection of in urine, 795
properties of, 104
in urine, 794
Nucleohistone, 307
in blood, 316
in urine, 795
of thymus, 367, 368
Nucleon, 578, 620, 666
Nucleo proteins, 92, 167, 174–177, 252

Nucleo proteins, action of trypsin on, 508
cleavage products of, 177–195
of blood, 258
of milk, 653
of the spleen, 369
peptic digestion of, 472
Nucleosidases, 182
Nucleosides, 180, 211
Nucleosin, 195
Nucleotides, 179
mono-, 179
poly-, 179
Nucleotin, 179, 182
Nucleotoprotamines, 174, 623
Nutritive need of man, 916
requirement, 908
Nylander Almén's test, 215
sugar test, 802

Obermayer's indican test, 729
Obermüller's cholesterin reaction, 445
Oblitine, 578
Ochronose, 550
Octodecapeptide, 85, 87
Oedematous fluid, and its constituents, 363
Oesophageal fistula, 459
Oil-turpentine, behavior in animal body, 749, 784
Olein, 236
mono and tri enzymotic synthesis of, 60
Oligæmia, 340
Oligocythæmia, 340
Oliguria, 771
Olive oil, absorption, 544
effect upon bile secretion, 415
Onuphin, 171
Oocyan, 636
Oorodein, 636
Opalisin, 652
Opium, passage of, into milk, 670
Organic phosphorous compounds in urine, 757
in urine, 762
sulphur compounds in urine, 752, 765
Orcin-hydrochloric acid test, 209, 223
Orcin, test for pentoses in urine, 817
Oriental bezoar-stone, 524
Ornithine, 85, 162, 163
Orthonitrobenzaldehyde, fate of in the body, 784
Orthonitrophenylpropiolic acid, test for dextrose, etc. See Acid nitrophenylpropiolic.
Orthonitrotoluene, fate of, in organism, 785
Osamines, 204
Osazones, 202, 203, 215
Osimines, 201
Osmometer, 16
Osmosis, 5
Osmotic pressure, 1–13
determination of, 16
Osmotic pressure, effective, 12
effect of various substances upon, 17
electrolytes, abnormal, due to ionization,
in higher animals, 13
in lower sea animals, 13
in lymph, 13
in milk and bile, 13
non-electrolytes, 4
of animal fluids, 12
of colloids, 16–18
of the blood, in selachii, 13
in saliva, 13
in urine, 13
Osones, 203
Ossein, 551
Osseoalbuminoid, elementary composition of, 551
Osseomucoid, 172, 551
elementary composition of, 551
Osteomalacia, 556, 557
Osteosclerosis, 556
Otoliths, 619
Ovalbumin, 633–635
cleavage products of, 106
preparation of, 634
Ovaries, 623
cysts in, 624
Ovin, 631
Ovoglobulin, 84, 633
Ovokeratin, 114
Ovomucin, 634
Ovomucoid, 171, 633, 635, 636
properties, 635
preparation, 635
Ovovitellin, 84, 91
composition of, 628, 629
preparation of, 630
Ovum, 623, 628
Oxamide, 83
Oxidases, 188
artificial, 873. See also Enzymes
Oxidation in animal tissues, theories of, 42
in the organism, 850–877
Oxidones, 874, 875
Oxydase, 47, 48
Oxygen absorption, calculation of, 889
activation, 4–8
tension in blood, 859–861
tension, regulation of in organism, 868
Oxygenases, 872
Oxyhæmatin, 289–291
Oxyhæmocyanin, 299
Oxyhæmoglobin, 276, 278–282
absorption bands of 281, 282
action of trypsin on, 508
effect of gastric juice on, 473

Oxyhæmoglobin, law of dissociation of, 859
preparation of, 282
See also Blood pigments.
Oxyphenylethylamine, 82
Oxyproline, 85, 106, 107, 125, 155
Oxyprotein, 83
Oxypurine, 190
Oxypyrimidine, 195
Oxyquinolines, fate of, in organism, 785
Ozone, occurrence in the organism, 4

Palmitin, 235
Pancreas, 494–510
importance of, to absorption of carbohydrates, 535
Pancreatic diastase, 501
gland, constituents of, 495
juice, 494–501
action on fats, 502
action of, on starch, 226
amount secreted, 500
constituents of, 500
excitants for the secretion of, 498
properties of, 500
lipase, 57. See also Enzymes.
secretion of, 496
rennin, 509. See also Enzymes.
secretion, human, 500, 501
Papain, 51
Paracasein, 650
Parachymosin, 474, 567
Paracresol, 515
formation in putrefaction, 515, 723, 724
Paraglobulin, 258
Parahæmoglobin, 280
Parahistone, 109
Paralbumin, 624, 625
Paraminophenol, 779
Paramucin, 626
Paramyosinogen, 568, 570, 571
Paranuclein. See Pseudonuclein
Paraovarial cysts, 627
Paraoxypropiophenone, 785
Parapeptone, 472
Parathyroids, 377
Paraxanthine in urine, 712, 713, 714
Parenchymatous organs, action of trypsin on, 508
Parenteral canal, introduction of protein by way of, 533–541
Parotid, 448
saliva, 450
Peaglobulin, 84
Pectin bodies, 229, 231
Pemphigus chronicus, 359
Pennacerin, 846
Pennatulin, 123
Pentacrinin, 844
Pentamethylenediamine, 47. See Cadaverin
Pentapeptides, 85
Pentosamines, 220
Pentosans, 207
Pentoses, 197, 198, 199, 207–211
in urine, 816
quantitative estimation, 209
test, orcin-hydrochloric, 209
tests for, 209
Pentosides, 180
Pentosuria, 816
Penzoldt's test for acetone, 823
Pepsin, 57, 64, 466–476
action on proteins, 468–473
characteristic property of, 468
digestion of proteins, products of, 472
digestion, effect of foreign substances upon rapidity of, 470
digestion, rapidity of, 471
digestion of various bodies, 472, 473
mother substances of, 477
nature of, 467
occurrence of, 466
preparation of, 468
properties of, 467, 468
quantitative methods for, 469, 470
relation to rennin, 475
test for, 469
testing for, in gastric juice, 487
Pepsinogen, 477
Peptases, 503. See also Enzymes.
Peptic-glutin peptone, 135
peptones, 135
zymolysis, effect of bile upon, 511
Peptidases, 48
Peptides, 68, 85–89, 93, 126, 127, 156
behavior toward enzymes, 8, 266, 492, 510, 511. See Polypeptides.
in urine, 755
relation to alcaptonuria, 735
relation to urea formation, 777
Peptinuria, 791
Peptochondrin, 549
Peptoid, 507
Peptone-plasma, 251
Peptones, 92, 127–132
anti-, 132
as foodstuff, 912
carbamino reaction with, 167
from peptic digestion 472
in urine, 791
properties of, 131
quantitative estimation, 138
separation from proteoses, 136, 138
Percaglobulin, 637
Pericardial fluid, 356, 357
analysis of, 356
Perilymph, 619
Peritoneal fluid, 357

Permeability, causes for,
of blood corpuscles, 7
Overton's solubility theory, 10
shortcomings of, 10, 11
Traube's solution tenacity theory, 11
Peroxidases, 872. See also Enzymes.
Peroxydase, 50
Perspiration, 847–849
circumstances influencing, 847
constituents of, 848
properties of, 847
Pettenkofer's method for determining respiratory change, 869
Pit test for bile-acid, 798
test for bile, 418
Pfaundler's method of precipitating urine, 681
Phaseomannite, 579
Phenol, 515
as precipitant of proteids, 98
in urine, 515, 723, 726, 785, 787
Phenol-oxidases, 875
Phenols, 117, 723
fate of, in organism, 775, 784
Phenylalanine, 85, 109, 114, 151, 152
amounts in proteins, 106, 107, 113, 115, 125
Phenylethylamine, 82
Phenylisocyanate compounds of amino acids. See various Acids, amino.
peptone, 138
Phenylmethylketone, 785
Philothion, 876
Phlebin, 276
Phosphates, importance of, in metabolism, 902
in urine, 761–764
in urine, quantity of, 762
See also Mineral substances.
Phosphatides, 239–249, 265, 586
in adrenal bodies, 377
in bile, 435, 439
in kidney, 673
in liver, 385
in yolk, 630
Phosphaturia, 763
Phosphoglycoproteins, 174, 177
Phosphoproteins, 91, 92, 93
properties of, 104
Phosphorus compounds, action on bile, 437, 441
action on blood, 252, 254
action on urea elimination, 679, 685
organic, in urine, 756
metabolism, 875, 876, 894
Phosphorus-containing urinary constituents, 756, 875
elimination in organism, 883
elimination in relation to nitrogen elimination, 762, 763
elimination in relation to purine metabolism, 763
Phosphatides, properties of, 241
Photomethæmoglobin, 284
Phrenin, 612
Phrenosin, 609, 610, 611
Phyllocyanin, 295
Phylloerythrin, 435
Phylloporphyrin, 276, 295
Phymatorhusin, 841
in urine, 799
Physical chemistry in biology, 1
Physiological oxidation processes, 871–877
salt solution, 7
Phytase, 579
Phytin, 579
Phytosterines, 446
Picolin, behavior of, in animal body, 784
Pigments, fate of, in organism, 787
medicinal in urine, 801
of bile, 427, 433, 435, 436
of birds' eggs, 636
of blood, 275, 298
of blood serum, 268
of butterflies, 844
of corpora lutea, 297, 623
of eye, 614, 616
of feathers, 843, 844
of human skin, 840–843
of liver, 384
of lobster, 638, 844
of lower animals, 299, 844
of lungs, 870
of muscle, 571
of placenta, 641
of pus, 366
of skin, 840–844
of stones, 441
of urine, 733, 734, 740, 748, 798, 799
of yolk, 631
Pilocarpine, effect upon elimination of CO_2 in stomach, 485
effect upon elimination of uric acid, 700
effect upon secretion of intestinal juice, 490
effect upon secretion of saliva, 457
Piperdine-glycosuria, 403
Piqûre, 402
Piria's test for tyrosine, 153
Placenta, 641
Plant cells, 5
osmotic experiments with, 5
gums, 226
Plasmolysing power of differently constructed salts, 6
Plasmolysis, 5

Plasmolysis, substances bringing about, 5
Plasmolytic experiments with animal cells, 7
Plasmoschisis, 314
Plasmozym, 319
Plastein, 59
Plasteines, 135
Plastin, 178
Plattner's crystallized bile, 396
Pleural fluid, 357
Pnein, 874
Pneumonic infiltrations, solutions of, 18, 368, 869
Poikilocytosis, 342
Polycythæmia, 340
Polynucleotides, 179
Polypeptide-like bodies in urine, 756
Polypeptides, 86–91, 92. See Peptides.
action of trypsin on, 509
cleavage of, by enzymes, 62
E. Fischer's synthetical preparation of, 88
methylated, 88
syntheses of, 86
synthetically produced, 87
sulphur containing, 88
synthetic, comparison of properties with natural proteins, 90. See also Peptides.
Polyperythrin, 844
Polysaccharides, colloid, 226–231
Polyuria, 771
Potassium in urine, 766. See also Mineral substances.
Präglobin, 307
Precipitation, Traube's membrane, 1
Precipitins, 66
Preglobulin, 315, 368
Preputial, secretion of skin, 846
Proenzymes, 51
Prolamine, 78
Prolamins, 106
Proline, 85, 109, 111, 154, 155
amounts in proteins, 106, 107, 113, 115, 125
Propepsin, 477
Propeptone, 126
Propylalanine, 85
Propyl benzene, behavior in animal body, 778
Propylene glycol, relation to glycogen formation, 394
Prosecretin, 463, 499
Prostate, secretion of, 621
Prostatic concrements, 172, 611, 623
Prosthetic group, 174
Protagon, 605, 606–608, 609
cleavage products of, 607
elementary composition of, 606
nature of, 606, 607
preparation of, 608
properties of, 608
Protamine nucleate, 623
Protamines, 91, 92, 93, 108
hexone bases in, 165
preparation of, 112
properties of, 109–112
Proteans, 93
Protease, 47, 48
alpha retardation of, 63
Proteases, 48
Protective colloids, 37, 44, 131
Protein, absorption, effect of cellulose on, 531
catabolism, 907, 908, 909, 911
importance of sulphur in, 882, 883
in active muscle, 594
in starvation, 894
destruction of, during work, 595
ochrome, 155
requirement, 933–936
lower limit, 915]
sparers, 913–915
substances, diarginylalanine, 111
diarginylproline, 111
diarginylserine, 111
diarginylvaline, 111
Proteins, 38, 77–195
absorption of, 525–531
acid, 125
action of neutral salts on, 98
action of nitrous acid upon, 79
action of pepsin on, 468–473
action of trypsin on, 505, 507
adsorption compounds, 95
adsorption of, 97
albuminates, 104
albuminoids, 112
albuminous, 102–138
albumins, 106
albumose, alkali, 127
alcohol soluble, 92
alkali, 126
amino acids in, linking of, 90
amounts in blood of different animals, 328
analysis, 118
atmidkeratin, 113
atmidkeratose, 113
Bence-Jones, 792
byssus, 122, 123
carbon dioxide binders in blood 855
casein, 106
chitin, 122
chondrin, 121
chondro-, 168, 172–174
chromo-, 167
circulating, 908–910
classification of, 91–93
cleavage products, 93, 106
clupeine, 110
coagulated, 93
coagulation of, 96
collagen, 118, 119
color, reaction for, 99

Proteins, composition of, 77, 94
compound, 92, 167–177
conchiolin, 122, 123
conjugated, 92, 93
cornein, 122, 123, 124
cornicrystalline, 123
crystalline, 94
cyclopterine, 110
cyprinine, 110
derived, 93
deutero, 120
deuteroelastose, 117
effect on glycogen content of liver, 394, 397
elastin, 116–118
fate of, in organism, 886
fattening, 919
fibrin, 106
fibroin, 122, 124
foodstuff purposes of, 917, 918
forms of nitrogen in, 77, 78
gelatin, 118, 119–121
gelatin-peptones, 120
gelatose, 120
gelatoses, 120
globan, 104
globulins, 103–104
gluco-, 167, 168–174
glucoproteins, phosphorized, 105
gluteins, 119
glyco-, 92, 167, 168–174
gorgonin, 123
helico-, 174
hemicollin, 120
hemiélastin, 117
hordein, cleavage of, 107
hydrolysis of, 81
ichthylepidin, 122
in amniotic fluid, 642
in aqueous humor, 361
in ascitic fluids, 359
in bone, 551
in bone marrow, 554
in brain, 604
in cartilage, 546
in cerebrospinal fluid, 360
in chyle, 347
in connective tissue, 544
in crystalline lens, 617, 618
in cystic fluids, 625–627
in dead muscles, 567
in fat globules, 647
in horn substance, 837
in hydrocele and spermatocele fluids, 359
in kidneys, 672
in liver, 382, 383
in lungs, 870
in lymph, 346, 348
in lymphatic glands, 366
in mammary glands, 643
in milk, 647–654
in milk plasma, 647
in muscles, 566–572
Proteins, in muscle-plasma, 569, 570
in pancreatic gland, 495
in pericardial fluid, 356
in placenta, 641
in prostate secretion, 621
in pus corpuscles, 364, 365
in pus-serum, 363
in retina, 615
in salivary glands, 451
in sebum of skin, 844
in semen, 620
in smooth muscle, 602
in spermatozoa, 623
in sputum, 871
in synovial fluid, 362
in testes, 620
in thymus, 366, 367
in transudates and exudates, 353, 354
in urine, 787-795
in urine, detection of, 788
in urine, quantitative estimation of, in urine, 793
in veins, 336
in venous blood, 336
in white of egg, 633–636
in yolk, 628–630
iodo, 123
keratins, 112–116
koilin, 115
lecithalbumins, 105
lecitho, 92
Lieberkühn's solid alkali albuminate, 126
metabolism calculation of, 889
metabolism of, in starvation, 893, 894
metabolism with food rich in proteins, 906, 913
method of synthesis, 86
membranins, 617
modified, 96
molecular weight, 98
mucins, true, analysis of, 168–172
mucinoids, 171
mucoids, 171
native, 96
Neubauer and Rohde's test, 100
nuclei of aliphatic series, 85
nuclei of carbocyclic series, 85
nuclei of heterocyclic series, 85
nucleo, 92, 105, 167, 174–177
nucleo albumins, 104, 105
ovokeratin, 114
ovovitellin, 105, 106
parenterally introduced, absorption of, 525
pennatulin, 123
phospho, 92, 104
phosphoglycoproteins, 174, 177
phosphorized importance of, in metabolism, 902

Proteins, precipitation of, 95, 96, 98, 99
preparation, 118, 121
prolamine, 106
properties of, 94–96, 107, 108, 119
protamine, constitution of, 111
protamines, 109–112
proto-, 120
protoelastin, 117
protones, 110
pseudomucins, 171
pseudonucleins, 104
putrefaction of,
putrefactive products of, 82
quantitative estimation, 101
reaction of, 91
reactions, precipitation, 98
reticulin, 121, 122
salmine, 110
salting out of, 95
scombrine, 110
semiglutin, 120
seralbumin, cleavage products of, 106
serglobulins, cleavage of, 106
sericin, 122, 124
simple, 91, 92, 93, 94
cleavage products of, 125–195
skeletins, 122
constitution, 122
source of muscular energy, 595–597
spongin, 122, 123
sturine, 110
sulphur content,
syntheses from amino acids, 529, 530
synthesis of, in organism, 530
synthetically formed, 59
tests for, 99, 100, 101
tissue, 908–910
xanthoprotein reaction, 99
zein, cleavage products of, 107
Proteose-like substances in blood serum, 264
Proteoses, 92, 127–132
absorption, 534, 538
as foodstuff, 912
carbamino reaction with, 167
detection of, in urine, 792
deutero, 130
dys, 130
from peptic digestion, 472
gluco-, 134
hetero. 130, 134
in blood, 262, 263, 534, 535
in intravascular coagulation, 325
in lungs, 870
in stomach, 483, 484
in urine, 791
primary, 130
properties of, 131
Proteoses, proto-, 130, 134
quantitative estimation, 138
secondary, 130
separation from peptones, 136, 138
syn-, 134
Prothrombin, 255, 256, 257, 312, 314, 315, 317
in circulating plasma, 318
Protocaseoses, 129
Protoelastose, 117
Protogelatose, 119
Protogen, 126
Protokyrins, 138
Protones, 110
Protoplasm, 5
Protoproteose, 131, 133, 135
Protosyntonose hexone bases in, 165
Proto-toxoids, 71
Pseudocerebrin, 611
Pseudoglobulin, 259
Pseudohæmoglobin, 283
Pseudomucin, 171
beta, 625, 626
detection of, 626
hydrolytic cleavage products of, 626
Pseudonuclein, 629, 651
Pseudonucleins, 104, 177
Pseudopepsin, 464, 489
Pseudoxanthine, 578
Psittacofulvin, 843
Psylla-alcohol, 845
Ptomaines, 46, 82
in urine, 757, 758
Ptyalin, 48, 456–460
action of, 456, 457
best reaction for, 457
condition of, in the intestine, 510
effects of foreign substances upon, 457, 458
preparation of, 456
Purine bases, 186–193, 267, 715
detection of, 193
in active muscles, 594
in adrenal bodies, 377
in spleen, 370
preparation of, 193
quantitative estimation of, 715
transformation into uric acid, 702, 703
bodies, in pus-corpuscles, 364
composition of, 187
oxidases, 875. See Enzymes.
skeleton, 186
structural formula of, 186
tri-oxy-, 2, 6, 8, 187
Purines, in lymphatic glands, 366
in thymus, 368
in thyroid gland, 373
Pus, 363
cells, analysis of, 365
corpuscles, 364–366

Pus, constituents of, 364
in urine, 799
serum, 363
analyses of, 363, 364
analyses of ash of, 364
Putrefaction, 43, 46
factors influencing, 516–520
in intestines, importance of, 517
preventive substances for, 518
Putrefactive products, absorption of, 515
conjugation of, 515
elimination of, in urine, 515
in large intestine, 515
Putrescine, 47, 82, 162
in urine, 827
Putrine, 47
Pyin, 363, 366
Pyloric secretion, 478
Pyocyaneus protease, 57
Pyocyanin, 366
Pyogenin, 364
Pyosin, 364
Pyoxanthose, 366
Pyrazine, formation from glycocoll, 220
Pyridine, fate of in organism, 787
structural formula of, 186
Pyrimidine, 193
bases, 177, 178, 193–195
Pyrin, 357
Pyrocatechin in urine, 727
Pyrrol derivatives, 154, 158, 276, 290, 740
Pyrrolidone-carboxylic acid, 113

Quercinite, 581
Quercite, 394
Quinidine, as catalyst, 60
Quinine, as catalyst, 60
effect upon spleen, 375
Quotient, $\frac{Cal}{L.CO_2}$, 888
$\frac{Cal}{L.O_2}$, 888
$\frac{Cal}{N}$, 892
C to N, 771
flesh, 602
nitrogen to homogentistic acid, 735
respiratory quotient, 412, 563, 593, 879, 887, 918
urea to nitrogen, 777, 877
calorific urine, 892
$\frac{CO_2}{O}$, 887
$\frac{C}{N}$, in feces, 584
Quotient, $\frac{C}{N}$, in urine, 771
$\frac{N}{C}$, in urine, 883
$\frac{N}{S}$, in urine, 882
calculation of, 889
in starvation, 895
respiratory, 887, 927, 928

Rachitis, 556, 557
Reaction, of a solution, determination of 74
velocity, 32
Reactivation enzymes, 52
Receptors, 67
Reducing substances in urine, 749–752
Reductase, 47, 48
Reductases, 876, 877
Reductions in animal body, 876
Reductonovaine in urine, 758
Regnault-Reiset's method for determining respiratory exchange, 869
Reichert-Meissl's equivalent for fats, 238
Rennet, 649
Rennin, 48, 49, 57, 474–476
action of on charcoal, 62
anti, 64, 69
occurrence of, 474
pancreatic, 509
preparation of, 475
properties of, 475
relation to pepsin, 475
retardation of, 63
retarding substances for, 474
testing for in gastric juice, 487
See also Enzymes.
zymogen, 474
Resacetophenone, 785
Resins, fate of in organism, 787
Respiration, 850–877
apparatus, experiments with, 889, 890
external, 858
gas tension in blood, 859–868
importance of hæmoglobin in oxygen-carbon dioxide exchange in blood, 853
internal, 858, 867
mechanism of carbon dioxide elimination, 852, 853
processes taking part in the gas exchange, 858
Respiratory exchange, methods for determining, 868–870
quotient in active muscle, 596
in diabetes 412–413
Rest carbon, 268
Rest nitrogen, in serum, 267
in stomach, 483, 484
and work, effect on metabolism, 926–928

Reticulin, 92, 121, 544
composition of, 121
in lymphatic glands, 336
properties of, 121
preparation of, 122
Retina, constituents of, 615
pigments of, 615–617
Reynold's test for acetone, 823
Rhamnose, 208
relation to glycogen formation, 394
Rhodophan, 617
Rhodopsin, 615
Ribose, structural formula for, 199, 211
d-Ribose, 178
Ricin, 470, 505
in testing pepsin, 470
Rigor mortis, 601
of muscles, 588–591
Ringer-Locke's solution, 75
Ritthausen's method of determining proteins in milk, 656
Roch's test for proteid, 995
Rosenbach's bile pigment test, 799
urine test, 827
Rotation, specific, 199
Rovida's hyaline substance, 274, 302, 367
Rubner's reaction for dextrose, 215
sugar test in urine, 809

Saccharase, 48, 49, 57. See also Enzymes.
action of charcoal on, 63
retardation of, 63
Saccharides, mono, 197–218
di, 197, 223–226
poly, crystalline, 197
poly, colloid, 197, 226
tri, 197–226
Saccharose, 223, 224, 225. See Sucrose.
Sachsse's reaction for dextrose, 295
Sahidin, 609
Sahli's hæmometer, 299
Saliva, the, 451–460
action of, in stomach, 480
on starch, 226
chorda, 452
constituents of, 453
diastatic power of, 71
gases of, 857
human, quantitative composition of, 458
quantity secreted, 459
mixed buccal, constituents of, 455
paralytic, 452
parotid, 453, 454
physiological importance of, 459, 460
properties of, 453, 454, 455
sublingual, 453
submaxillary, 452
sympathetic, 452
Salivary concrements, 460
Salkowski's creatinine reaction, 696
reaction for chloesterin, 444
Salmine, 110
Salt action on enzymes, 70–76
glycosuria, 402
plasma, 251
Samandarin, 846
Sandmeyer's method of inducing pancreas diabetes, 405
Santonin, elimination of in urine, 787, 800
Sapokrinin, 499
Saponin, 273, 446, 447
Sarcolemma, relation to permeability, 9
Sarcomelanin, 841
Sarcosine, fate in organism, 786
Sarkine. See Hypoxanthine, 190
Sarkosine, 776
Schalfejeff's, hæmin, 292
Scherer's inosite test, 578
Schiff's reaction, 686
reaction for cholesterin, 445
Schreiner's bases, 621
Schutz-Borrissow's rule of ferment action, 54, 471, 476, 503, 506
Schütz's rule, 58
Schweitzer's reagent, 231
Scleroproteins, 93
Sclerotic, 619
Scombrine, 110
Scombron, 108
Scyllite, 370, 581
Scymnol, 418
Sebelien's method of determining proteins in milk, 656
Sebum of skin, 844
Secretin, 463, 492, 498, 499
Secretion enzymes, 52
of prostate, 621
Sedimentum lateritium, 708
Segregation, 52
Seliwanoff's reaction for levulose, 218, 815
Semen, 620, 621
Semicarbazide, poisoning therewith, 718
Semiglutin, 119, 120
Seminose, 216, 217
Semi-permeable, membrane, 1
Senna, elimination of coloring matter of, in urine, 787, 802, 827
Sensibilizators, 69
Sepia, 381, 841, 843
Sepsine, 47
Seralbumin, 84, 91, 252, 261–264
crystalline, 262
composition of, 262
elementary composition of, 263
preparation of, 262, 263
properties of, 262, 263. See also Proteins.
quantitative estimation of, 263
Serglobulin, 84, 91, 252, 258–261
detection of, 261
elementary composition of, 263

Serglobulin, para-, 258
preparation, 259, 261
properties, 260
quantitative estimation, 261.
See also Proteins.
Sericin, 92, 122, 124
Serine, 106, 107, 111, 113, 115, 125, 145
iso-, 146
Serolin, 265
Seromucoid, 261, 264
Serosamucin, 354
Serum, casein, 258. See Serum globulin.
normal. See Blood serum.
Sham feeding, 459, 464, 821
Shark, bile, 416, 433, 681
urea, 334, 433, 681
Shell, membrane of hen's egg, 636–641
Side-chain theory, 71
Siegfried and Zimmermann's method for phenol-sulphuric acids, 726
Siegfried's carbamino reaction, 166
Silk gelatin, 123, 124
Sinistrin, animal, 174
Skatol, 46, 82, 117, 157–159, 515
522, 723, 732, 843
fate of in organism, 786
Skatosine, 159
Skatoxyl, 724
tests for, 732
Skeletins, 122
composition of, 122
Skin, 837–849
exchange of gas through, 849
horn formations of, 840
human, pigments of, 840–843
melanins of, 841
perspiration of, 847–849
pigments of 840–844
sebum of, 844
secretion, preputial, 846
secretions of, 837–849
of various animals, 846
Smegma præputii, 844
Snake poison, effect upon blood, 255, 310, 325
Soaps, 265
in chyle, 347
Sodium alcoholate as a saponification agent, 234, 237
compounds, division among form elements and fluids, 22, 23
in urine, 766. See Mineral substances.
Solanin, 273
Solution tenacity, 10
in relation to Osmosis Relationship to surface tension, 11
Sorbin, 218
Sorbinose, 218
Sorbite, 198
Sörensen's formol titration for amino-acids, 166
Specific dynamic action, 918
Speck's method for determining respiratory exchange, 869
Spermaceti, 238
oil, 238, 239
Spermatin, 623
Spermatocele fluids, 359, 360
Spermatozoa, 620, 622, 623
properties of, 622
constituents of, 622
Florence's test for, 621
osmotic phenomena with, 8
Spermine, 621, 622
crystals, Böttcher's, 621
Sphygmogenin, 378
Sphyngomyelin, 608, 609
Sphyngosin, 611
Spiegler's test for proteids, 789
Spinal marrow, 614
Spirographin, 171
Spleen, 369–373
analyses of, 372
constituents of, 370
pathological processes in, 372
physiological functions of, 372
uric acid formation in, 702, 703
Spongin, 92, 122, 123
iodo-, 123
Sponginoses, 122
Spongosterin, 449
Sputum, 871
form constituents of, 871
Stachyose, 226
Starch, 226–228
artificial, 227
cellulose, 227
granulose, hydrolysis, 228
gum, 229
soluble, 227
Starvation, bone, catabolism in, 896
catabolism of fat in, 894
gas exchange in, 895
loss in weight of different organs in, 896
metabolism, 892–906
metabolism of fats in, 894
mineral substances in, 895, 896
nitrogen content of urine in, 897
nitrogen elimination in, 893, 894
protein in, 894
metabolism in, 893, 894
time interval to death, 892
urea content in, 897
urinary constituents in starvation, 899
water in, 895
Steapsin, 476, 501–503
effect upon, of bile, 511
Steapsinogen, 478
activation of, by bile, 511
Stearine, 234, 235

Steensma's modification of Gunsburg's test, 487
Stenson's test, 590
Stentorin blue, 844
Stercobilin, 428, 429, 529, 743
in feces, 532
Stercorin, 448
Stethal, 239
Stoke's reduction fluid, 282
Stomach, absorption of cleavage products in, 484
action of salivary diastase in, 460
contents. See Chymus.
digestion and absorption of various food in, 513, 514
fistulas, 365, 460
gases in, 485, 486
glands, 458
lactic acid fermentation in, 485
movement of food in, during digestion, 479, 480
movement within, during digestion, 479
self-digestion of, 486
Stone-cystine, 148, 150
Stroma-fibrin, 275
of blood corpuscles, 712, 273
Stromata, 273, 274
Strychnine, and glycogen transformation, 391, 394
Sturgeon, spermatozoa of, 110, 181
Sturine, 110
hexone bases in, 165
Stutz's test for proteid, 794
Subcutaneous oedema, fluid of, 362
Sublingual glands, 448
saliva, 450
Submaxillary glands, 446
Submicrons, 19
Substrate, 47
"Sucre immediat," 331
"Sucre virtuel," 331
Sugar, amounts in blood of different animals, 328
amounts in blood, 331
detection of in urine, 802–808
fats, in liver, 412, 413
gelatin, 139
glycogen, 393, 395
in aqueous humor, 361
in ascitic fluids, 359
in blood, 329, 330
in blood serum, 265
in cerebrospinal fluid, 360
in lymph, 346
in transudates and exudates, 355
in urine, 802–818
in venous blood, 336
proteins, in liver, 409–411
Sulphæmoglobin, 286
Sulphate quantitative estimation of, in urine, 765
Sulphates, amounts in urine, 765
Sulphates in urine, 764–766. See also Mineral substance.
ethereal in urine, 765
quantitative estimation of, 765
Sulphatide, 669
Sulphocyanides, occurrence in urine, 752, 775
Sulphonal intoxication, urine, 294, 797
Sulphur, compounds in urine, 752, 753
elimination, importance of in protein metabolism, 882, 883
in proteins, 78, 79. See also various proteins.
in urine, 752, 874, 875, 880
methæmoglobin, 286
Sulphuretted hydrogen in urine, 753
Suprarenin, 387
Surface tension, in relation to osmosis. 10
Suspension colloids, precipitation of, 70
Suspensoids, properties of, 15
Sympathetic saliva, 449
Synovia, 360
Synovial cavities around joints, fluid in, 360
analysis of, 362
fluid, 362
Synoviamucin, 362
Synovin, 362
Synproteose, 135
Syntonin, hexone bases in ,165
Syntoxoids, 71

Tænia, 389
Talose, 211
Tartar, 460
Tatalbumin, 632
Taurine, 150, 151
fate of in organism, 786
Tears, 619
Teichmann's crystals, 292, 796
Tendon mucoid, 544
elementary composition of, 551
Terpenes, fate of in organism, 785
Testes, 620
Tetanolysin, 72
Tetanotoxine and gastric juice, 485
Tetraglyclyglycine, 85, 510
Tetramethylene diamine, 47. See Putrascine.
Tetrapeptides, 85, 87, 89, 510
Tetronerythrin, 843, 844
Tetroses, 197
Theobromine, 187
Theophylline, 187
Thiophene, behavior in animal body, 784
Thiotolene, 784
Thrombin, 48, 256, 315, 317, 324
action of, 256
formation of, 318
preparation of, 257
properties of, 256
pro-, 256
Thrombogen, 318, 321
formation of, 322

Thrombokinase, 318, 319
Thromboplastic substances, 326
Thrombosin, 320
-lime, 320
Thrombozym, 321
formation of, 322
Thymine, 178, 181, 185, 195
Thymus, 366–369
analysis of, 369
constituents of, 368
functions of, 369
Thyreoglobulin, 375, 376
Thyreoproteid, 375
Thyroid, gland, 373–376
constituents of, 373
effect of extirpation of, 374
Tissue, connective, analysis of, 545
elastic analysis of, 545
fatty, 558–564
fibrinogen, 307
proteins, 908–910
Tissues, end products of oxidation in, 871
gas exchange in, 858–870
gelatinous, 545
mucous, 545
oxidation in, 858
physiological oxidation in, 871
theories for oxidation processes in, 871, 872
Tollen's reaction for pentoses, 209
Rorive test for levulose, 218
test for glucuronic acid, 223
Toluene, fate of in organism, 779
Toluylenediamine, poisoning with, 441
Tonus, chemical, 590
Tooth, cement, 557
dentin, 557
enamel, 557
structure, 557
Toxin, cysts, 69
Toxins, 46, 66
a method of measuring, 67
Toxoid, 67
proto, syn, epi, 67
Toxons, 67
Toxophore, group, 67
Tradescantiadiscolor, 6
Transfusion, fluid for mamallian heart, 72
composition of, 72, 73
Transudates and exudates, constituents found in, 353–355
distinguishing feature in, 356
formation of, 352, 353
osmotic pressure in, 356
pathological, gases of, 857
reaction of, 356
Trichohyalin, 837
Triglycerides, 232
Triglyclyglycin, 85, 510
ethyl ester, 86
Trimethylamine in urine, 757
Trimethylvinylammonium hydroxide, 246. See Neurine.
Triolein, 233, 236
Trioses, 197
Trioxypurine, 2, 6, 8, 187. See Uric acid.
Tripalmitin, 233, 235
Tripeptides, 85, 509, 510
Triple phosphates, in urinary concrements 831–833
in urinary sediments, 831
Trisaccharides, 200
Tristearin, 233, 234
Trommer's test, 214, 793, 803
Trypsin 49, 57, 503–509
action of, 504, 505
action of charcoal on, 62
digestion action of foreign bodies on, 506
formation of, 497
preparation of, 504
properties of, 503
quantitative estimation of, 505
rapidity of, under various conditions, 506
retardation of, 63
tests for, 505. See also Enzymes.
upon other bodies, 508
Trypsinogen, 504
activation of, 496, 497, 498
activators of, 497
Tryptic digestion, 505
products, of 507
Tryptophane, 85, 111, 119, 155–157
Adamkiewicz - Hopkin's reaction, 157
amounts in proteins, 106, 107
effect of yeast upon, 206
quantitative, colorimetric method for, 157
Tryptophol, 157, 206
Tubo-ovaria, 628
Tunicin, 838, 839
Turacin, 843
Turacoverdin, 844
Turpentine, fate of in organism, 787
Tyrosinases, 875
Tyndall's phenomenon, 40
Tyrosine, 85, 109, 111, 119, 123, 124, 152–154, 267
amounts in proteins, 106, 107, 113, 115, 125
effect of yeast upon, 206
importance of in homogentisic acid formations, 737
tests for, 153
Tyrosol, 153, 206

Uffelmann's test for free lactic acid in gastric juice, 487
Umikoff's reaction, 663
Uracil, 178, 185, 194, 195
Weidel's reaction for, 194
Wheeler and Johnson reaction for, 194
Uranium salts, and glycosuria, 402

Urate, ammonium, in urinary sediment, 830
Urates, acid, in urinary sediment, 830
of urine, 674
Ultra violet rays, 50
Umbilical cord, 545
Urea, 267, 679, 691
compounds of, 687
creatinine, 682
formation, anhydride theory, 684
from ammonium salts, 683
from arginine, 682
in ascitic fluids, 359
in blood, 333, 334
in cerebrospinal fluid, 360
in lymph, 346
in muscles, 572
in the organism, 682
in transudates and exudates, 355
in veins, 336
mesoxalyl, 699
mother substances of, 682, 683
nitrate, 686
occurrence of, 679
other organs besides liver, 685
oxalate, 687
oxidation theory, 684
physiological significance of, 680
preparation of, 679, 687, 688
properties of, 686
quantitative methods for 689–691
quantity voided, 68
tests for, 686
Urease, 48, 829
Urein, 691
Ureotheobromine, 713
Urethane, 692
Uric acid stones, 832
Uricolysis, 704
Uridine, 180, 185
Urinary calculi, 828, 829, 832–836
calcium carbonate, 834
calcium oxalate, 833
chemical investigation of, 834–836
cystine, 834
fibrin, 834
phosphate, 833
scheme for chemical, analysis of, 836
uric acid, 833
urostealith, 834
xanthine, 834
pigments, 740–748
sediments, 674, 828, 829
acid hippuric in, 831
ammonium magnesium phosphate in, 831
ammonium urate in. 830
calcium carbonate in, 831
calcium oxalate in, 830
calcium phosphate in, 831
Urinary sediments, calcium sulphate in, 831
cystin in, 831
hæmatoidine in, 831
magnesium triphosphate in, 831
non-organized, 830, 831
triple phosphates in, 831
tyrosine in, 831
urates, acid in, 830
uric acid in, 830
xanthine in,
Urine, 670–836
abnormal color of, due to foreign substances, 787
acetoacetic acid in, 824, 825
acetone bodies in, 818–828
acetone in, 822–824
acid fermentation of, 829
alcaptonuric, test for, 739
alkaline due to ammonium carbonate, 676
fermentation of, 829
Almén's bismuth test for sugar, 803
amino-acids in, 827
ammonium urate calculi, 833
average composition of, 772
Bang's quantitative methods for sugar, 809–811
Bertrand's quantitative method for sugar, 811
Bial's test for pentoses, 816
bile-acids in, 799, 800
bile-pigments in, 800–801
casual constituents of, 772–787
cholesterin in, 827
cloudy, reasons for, 674
color of, 674
conjugated glucuronic acids in, 817, 818
cystine in, 827, 828
degree of acidity, 675
detection of acetone and acetoacetic acid in, 825
Bence-Jones protein in, 792
betaoxybutyric acid in, 826
bile-acids in. 800
blood in, 796
conjugated glucuronic acids in, 817, 818
fructose in, 814
hæmatoporphyrin in, 798
lactose in, 815
pentose in, 816
pigments in, 800, 801
proteins in, 787–781
proteoses in 791, 792
sugar in, 802–808

Urine, determination of acidity, 676,677,
ion acidity, 677, 678
specific gravity 678, 679
division of the nitrogen of, 681
Ehrlich's urine test, 826
end products of acids amino aromatic, 780–783
amino-acids, 774–776
benzene ring and homologues, 778, 779
fatty series, 773,774
heterocyclic compounds, 778–787
homocyclic compounds, 778–787
fat in, 827
fermentation test for sugar, 805
fructose in, 814, 815
gases of, 857
glucose in, 802–814
guaiac test for blood in, 796
hæmatoporphyrin in, 797, 798
Heller-Teichmann's test for blood in, 797
heptose in, 817
indican, 728
inorganic constituents of, 758–770
inosite in, 818
isolation of sugar from, 807
Kjeldahl's method for total nitrogen, 688
Knapp's quantitative method for sugar, 812
lactose in, 815
laiose in, 815
levulose in, 814, 815
maltose in, 815
medicinal coloring matter in, 801
melanin in, 799
microscopic investigation of blood corpuscles, 796
nitrogen content during starvation, 897
Nylander's test for sugar, 803
odor of, 674
organic physiological constituents of, 679–758
osmotic pressure of, 678
β-oxybutyric acid in, 825–827
passage of sugar into, 534
pathological constituents of, 787–828
pentoses in, 816
percentage division of nitrogenous substances, 681
phenylhydrazine test for sugar, 806
physical properties of, 674–679
physico-chemical methods in, 772
physiological constituents of, 679–787
Urine, pigments in, 798, 799
polarization of, for sugar, 807
pus in, 799
quantitative composition of, 770–277
determination of sugar, 808–814
determination of proteid, 793
determination of total nitrogen, 688
estimation of acetone in, 825
estimation of β-oxybutyric acid, 826
estimation of sugar by fermentation, 812
estimation of sugar by polarisation, 813–814
method for nitrogen in very small quantities of urine, 689
quantity of, 770
conditions affecting, 770
of solids excreted, 770, 771
reaction of, 674, 675
Rubner's test for sugar, 807
sedimentatum lateritium, 674
specific gravity of, 678
spectroscopic investigation for blood, 796
sugar in, 802–818
taste of, 674
transparency of, 674
Trommer's test for sugar, 802
urea content in starvation
Urinometer, 678
Urobilin, 429, 516, 740, 743–746
detection of, 747
formation of in organism, 744
in blood serum, 268
preparation of, 746
properties of, 745
quantitative estimation of, 747
tests for, 745, 746
Urobilinogen, 740
detection of in urine, 746
formation of in organism, 744, 746, 747
in blood serum, 268
preparation of, 746, 747
properties of, 746
quantitative estimation of, 747
Urobilinoids, 743
Urochrome, preparation of, 742
quantitative estimation of,742
Urochrome, 740, 741, 742
in urine, 755
Urocyanin, 741

Uroerythrin, 740, 748
tests for, 748
Urofuscohæmatin, 798
Uroglaucin, 741
Urohæmatin, 741
Urohodin, 741
Urohypertensin, 758
Urohypotensin, 758
Uromelanins, 741
Urophain, 740
Uropyrryl, 741
Urorosein, 741
in urine, 733
Urorubin, 741
Urospectrin, Saillet's, 797
Urostealith calculi, 834
Urotheobromine, 713
Urotoxic coefficient, 758
Urorubrohæmatin, 798
Uroxanthine, 728
Uterine milk, 642
Uterus, colloid, 627

Valine, 111, 140, 141
amounts in proteins, 106, 107, 113, 115, 125
Vanillin, behavior in animal body, 779
Van Slyke's method for amino acids, 79
Van't Hoff's rule, 201
Velocity coefficient, 34
Vernine, 178
Vernix caseosa, 845
Vesiculase, 621, 622
Viridinine, 47
Visual purple, 615–617
preparation of, 617
properties of, 616
regeneration of, 616
Visual red, 615
Vitali's pus-blood test, 796
Vitamine, 905
Vitellin, 628
cleavage products of, 106
Vitellolutein, 632
Vitellorubin, 632
Vitelloses, 130
Vitiatine, 578
in urine, 758
Vitreous humor, 617
Voit's normal average diet, 933
Volhard and Lohlein's quantitative method for pepsin, 470
Volhard's quantitative method for chlorides, 760

Walden's reversion, 89
Water, importance to life, 71, 72
lack of in food, 899
Wear and tear quota, 890, 917
Weidel's reaction for histidine, 160
xanthine, 190
Weiss' sparing theory, 396
Weyl's creatinine reaction, 695
Wheeler and Johnson reaction for Uracil, 194
Whey, 645
acid, 645
protein, 650
sweet, 645
Witch's milk, 665
Wool-fat constituents of, 846
Work affecting metabolism, 926–928
Worm, Müller's sugar test, 803

Xanthine, 86, 178, 187, 188, 189, 190, 712
in ascitic fluids, 359
in urinary calculi, 833, 834
in urinary sediments, 831
in urine, 710
para, 187
Seyler's reaction for, 190
Weidel's reaction for, 190
Xantho creatinine, 578
in urine, 698
Xantho melanin, 84
Xanthoproteic reaction for proteins, 99, 173
Xanthoprotein, 84
Xanthophan, 617
Xanthosine, 180
Xylene, fate of in organism, 779
Xyliton, 842
Xyloses, structural formula for, 198, 210

Yeast, 59, 62
action of, on glucose, 226
maltase, 266
Yoghurt, 654, 659
Yolk, constituents of, 628
fat of, 630
membrane, 628
mineral bodies of, 632
of hen's egg, 628
pigments of, 631
phosphatides of, 630
proteins of, 628–630
spherules, 628, 636

Zein, 77, 105, 163
Zinc, in the bile, 433
in the liver, 389
passage into milk, 670
Zooerythrin, 843
Zoofulvin, 843
Zooruhin, 843
Zuntz and Geppert's method for determining respiratory exchange, 869
Zymase, 41
Zymogen, rennin, 474
Zymogens, 51, 461
Zymoplastic substances in blood, 315

73

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